# ABSTRACTS OF COMMUNICATIONS

*The Forty-seventh Meeting of The Nutrition Society was held at the London School of Hygiene and Tropical Medicine, London, W.C. 1, on Saturday, 22 May 1948, at 10.30 a.m., when the following papers were read:*

## Some of the Difficulties in Determining Iron and Carbohydrates in Mixed Meals of Unknown Composition.

By C. G. DAUBNEY and J. KING, *Department of the Government Chemist, Strand, London, W.C. 2*

In the analysis of mixed meals of varied composition special precautions had to be observed which were based on previous examination of individual foodstuffs.

The colorimetric determination of iron required care to avoid pick-up of the metal from apparatus, dust, etc., while avoiding loss due to the formation of volatile halide, or the formation of a difficultly extractable ash. The *o*-phenanthroline method was adopted because it was known from past experience to be reasonably free from interfering factors, viz. effects due to time, temperature, concentration, pH, neutral salts, other cations and anions, etc., and the absorption curve of the coloured complex was suitable for colorimetry.

Substances formed on cooking are disturbing factors in the determination of carbohydrates. Sugars were determined by the Government Laboratory modification of the Lane & Eynon method (1923). Starch and its heat-degradation products presented great difficulties, and it was decided after examination of selected starchy foods that the differential acid-hydrolysis method of Fraps (1932) gave the most reproducible and reliable results. Other methods studied for comparison are summarized as follows: (1) hydrolysis by enzymes, (2) polarimetric, (3) colorimetric and (4) solubilization followed by precipitation as starch or its iodine compound.

### REFERENCES

Fraps, G. S. (1932). *J. Ass. off. agric. Chem., Wash.*, **15**, 304.
Lane, J. H. & Eynon, L. (1923). *J. Soc. chem. Ind., Lond.*, **42**, 32T.

## Evidence for the Need, by Certain Insects, for Three Chemically Unidentified Factors of the Vitamin B Complex.

By G. FRAENKEL, *Imperial College of Science and Technology, London, S.W. 7*

The mealworm, *Tenebrio molitor*, requires, in addition to all the chemically known

tested maximum positive effects on survival and growth are not reached at the same concentration. Optimal survival usually occurs at about half the concentration required for optimal growth. On some occasions optimal growth in the presence of a purified source of $B_T$ is significantly inferior to growth on diets which contain 1% yeast as the sole source of $B_T$. This would suggest the need, by *Tenebrio*, for a further unknown factor in yeast.

In recent experiments with another insect, *Tribolium confusum*, it has also become clear that a purified diet, which contains all the known B factors and 2·5% of a water-insoluble fraction of yeast as a source of biotin, is significantly improved by the addition of 1% yeast. With biotin substituted for the water-insoluble yeast fraction there is a further depression in the growth rate. There is so far no clear-cut evidence that *Tenebrio* requires this insoluble factor, nor of the identity of the other chemically unknown *Tenebrio* or *Tribolium* factors.

| | *Tribolium confusum* Average larval period (days) |
|---|---|
| Purified diet, containing nine known B vitamins including folic acid and biotin | 43·5 |
| Addition of 2·5% 'insoluble yeast' | 36·5 |
| Addition of 1% yeast | 29·1 |

REFERENCES

Fraenkel, G. & Blewett, M. (1947*a*). *Biochem. J.* **41**, 469.
Fraenkel, G. & Blewett, M. (1947*b*). *Brit. J. Nutrit.* **1**, i.

**Nutritive Value for Pigs of Breads Made from Flours of 70, 80 and 85% Extraction and Effect of Supplementation with Aneurin, Riboflavin and Nicotinamide.** By G. A. CHILDS and T. F. MACRAE, *R.A.F. Institute of Pathology and Tropical Medicine, Halton, Bucks*, and R. BRAUDE and S. K. KON, *National Institute for Research in Dairying, University of Reading*

The nutritive value of breads made from flours of 70, 80 and 85% extraction, prepared from the same grist, was determined in two separate experiments carried out in 1945 at Cambridge and at Shinfield. The diet fed consisted almost entirely of bread, the only supplements being cod-liver oil and a mineral mixture, and in some instances aneurin, riboflavin and nicotinamide to bring the amounts of these nutrients to the levels present in the 85% extraction bread. The individual feeding method was used with five replicates in Cambridge and five at Shinfield.

All animals increased in weight at a subnormal rate (see table). The nutritive value of bread made from 70% extraction flour was lower than that of bread made from 80 and 85% extraction. The B vitamins added had no marked effect. Protein appeared to be the limiting factor.

After 5 weeks deaths occurred among the pigs in Cambridge experiments. At Shinfield the first death occurred on the 20th day. Values given in the table are means for all replicates prior to the first death at each centre. At post-mortem the Cambridge

animals were found to have a marked enteritis affecting the ileum, *Clostridium welchii* (type A) being the micro-organism responsible for the disease. This organism is not normally pathogenic in the pig and is a normal inhabitant of the gut. The animals that died in the Shinfield experiments had pneumonia.

*Growth of groups of five pigs on breads made from flours of different extractions*

| Ration | Average weekly increase (kg.) | Food intake/kg. weight gained (kg.) |
|---|---|---|
| Cambridge experiment (after 35 days) | | |
| 70% Bread | 1·22 | 4·18 |
| 70% Bread + B vitamins | 1·25 | 4·09 |
| 80% Bread + B vitamins | 1·62 | 3·17 |
| 85% Bread | 1·55 | 3·30 |
| Shinfield experiment (after 19 days) | | |
| 70% Bread | 1·33 | 4·28 |
| 70% Bread + B vitamins | 1·47 | 3·87 |
| 80% Bread | 1·76 | 3·25 |
| 85% Bread | 1·65 | 3·53 |

**Malnutrition and Spinal Malformation.** By F. H. KEMP, E. EMRYS-ROBERTS and D. C. WILSON. *The Institute of Social Medicine, 10 Parks Road, Oxford*

There are many people in whom a radiological examination of the spine reveals changes which invariably cause some limitation of movement and may give rise to pain. They are not due to any one primary disturbance but may arise if the spine is of faulty construction or alinement or if it is damaged by trauma, occupation or ill health, and they tend to become more marked in later life. We have been particularly interested in those cases where the X-ray examination reveals degenerative changes of the intervertebral disks and lipping of the margin of the vertebras, a condition originally described by Scheurmann (1921, 1936).

We have been able to show that the occurrence of the disease is closely related to sustained malnutrition and that benefit results from improvement in diet. Such changes are not confined to children of the poorer classes, but they certainly occur less frequently in the children of the 'well-to-do'. Whenever we have seen a case in a middle-class family there has always been a history of sustained ill health or feeding difficulties (Kemp & Wilson, 1948). Other casual factors are postural disturbances and the rate at which growth occurs, and there is a strong probability that some factor closely related to fluorine in geological distribution is concerned (Kemp, Emrys-Roberts & Wilson, 1948). The possibility that fluorine is in itself partly responsible cannot be eliminated, since we know that fluorine may interfere with enzyme systems concerned in calcification and therefore cause changes in bone.

REFERENCES

Kemp, F. H., Emrys-Roberts, E. & Wilson, D. C. (1948). (In the Press.)
Kemp, F. H. & Wilson, D. C. (1948). *Brit. J. Radiol.* **11**, 449.
Scheurmann, H. (1921). *Z. orthop. Chir.* **41**, 305.
Scheurmann, H. (1936). *Fortschr. Röntgenstr.* **44**, 233.

**Haematuria in Pyridoxin-Deficient Rats.** By L. R. C. AGNEW, *Rowett Research Institute, Bucksburn, Aberdeenshire*

Haematuria was observed in hooded Lister Institute rats (Rowett Research Institute strain) weaned and maintained on a pyridoxin-deficient diet. The diet was composed of sucrose 73, vitamin-free casein (Glaxo Laboratories Ltd.) 18, margarine or lard 5 and salt mixture (McCollum 185 plus 0·221 g. potassium iodide/kg.) 4%. The following vitamins were incorporated in the casein: *i*-inositol 10, *p*-aminobenzoic acid 10, nicotinic acid 4, calcium pantothenate 2, aneurin 0·3 and riboflavin 0·3 mg./100 g. diet. Pyridoxin-HCl (0·3 mg./100 g.) was given only to the control animals. In addition, to each rat 30 mg. choline chloride was given by pipette on alternate days, 5 ml./250 g. margarine of Radiostoleum (British Drug Houses Ltd.) as source of vitamins A and D, and 2 mg. vitamin E (α-tocopherol) dissolved in arachis oil and given by pipette each week. In a preliminary experiment the haematuria was first noted in seven animals that had received the experimental diet for 34, 39, 40, 62, 63, 74 and 75 days. Haematuria was not observed in litter-mate inanition control rats (paired-fed or paired-weighed) or in *ad lib.* controls. The bleeding was transient (limits 1–13 days, usual duration 4–6 days), and never fatal.

In later experiments haematuria was noted earlier in the deficiency and lasted longer. It appeared in fifteen of nineteen (79%) pyridoxin-deficient rats and was not observed in twenty-two inanition and nineteen *ad lib.* control rats.

Haematuria has not been reported in any of the numerous recent papers from the United States on pyridoxin deficiency, and only Birch (1938) appears to have published on this subject. In his rats, however, bleeding appeared as a terminal symptom after many weeks on the deficient diet, and it is impossible to be certain that he was dealing with uncomplicated pyridoxin deficiency. Burr & Burr (1930) observed haematuria in essential fatty-acid deficiency, but it is unlikely that this was responsible in my experiments since the pyridoxin-deficient rats received 5% margarine or lard and haematuria did not appear in any of the controls. However, as there is evidence that pyridoxin deficiency and essential fatty-acid deficiency have certain common features, further work is in progress to explore this relationship.

REFERENCES

Birch, T. W. (1938). *J. biol. Chem.* **124**, 775.
Burr, G. O. & Burr, M. M. (1930). *J. biol. Chem.* **86**, 58.

**Combined Deficiency of Vitamin E and Protein in Rats.** By T. MOORE, *Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council*

The possibility that vitamin E may be related to protein metabolism has been suggested by several workers (Dam, 1944; Victor & Pappenheimer, 1945; Hove, 1946; Hove & Harris, 1947; Moore & Wang, 1947).

In the present experiments young female rats weighing 40–46 g. were given a basal diet deficient in vitamin E, at first containing 25% of casein. As they reached pre-

determined body-weights of 60, 70, 80 and 90 g. their casein intake was progressively reduced to 12, 6, 3 and 0% and replaced by more carbohydrate. These reductions were so timed that the rats stopped growing after about 6 weeks from the beginning of the experiment as they approached 100 g. in weight, with the dried yeast component of their diet as their only source of protein. At this point depigmentation of the incisor teeth was just beginning, although the teeth of control animals given tocopherol remained normal.

After about 10 weeks from the start of the experiment four groups of animals were selected. Rats in group 1 were each given 5 g. daily of a diet containing 40% of casein, and were also allowed sucrose *ad lib.* and daily doses of 1 mg. of tocopherol. Group 2 were fed similarly, but without tocopherol. Group 3 were given 5 g. daily of a diet with only 4% of casein, together with sucrose and tocopherol. Group 4 received the same treatment without tocopherol.

During the next 13 weeks equally good growth was seen in groups 1 and 2. Growth was poor in both the other groups, but was slightly better in group 3 than in group 4. Dental depigmentation was completely restored in groups 1 and 3, but teeth in group 4 became completely white, while in group 2 the top incisors were white and the bottom incisors brown. In these experiments, therefore, failure of growth was mainly due to lack of protein, and dental depigmentation to lack of vitamin E. Interaction between the two components was indicated by a slight improvement in growth caused by vitamin E when protein was lacking, and by some improvement in the teeth caused by protein when vitamin E was deficient.

At the end of the experiment the uteruses of all rats dosed with tocopherol were found to be normal in colour and in fluorescence, while those of rats not given tocopherol were slightly discoloured, and had abnormal fluorescence.

REFERENCES

Dam, H. (1944). *Proc. Soc. exp. Biol., N.Y.*, **55**, 55.
Hove, E. L. (1946). *Proc. Soc. exp. Biol., N.Y.*, **63**, 508.
Hove, E. L. & Harris, P. L. (1947). *J. Nutrit.* **34**, 571.
Moore, T. & Wang, Y. L. (1947). *Brit. J. Nutrit.* **1**, 53.
Victor, J. & Pappenheimer, A. M. (1945). *J. exp. Med.* **82**, 375.

**Haemoglobin Levels in Sedentary and Active Persons.** By E. W. Adcock, J. Kerr Brown, H. E. Magee and E. H. M. Milligan, *Ministry of Health, Whitehall, London, S.W.* 1

Within the range commonly regarded as 'normal' high haemoglobin values are generally regarded as indicative of more robust health than low values. Data we have obtained from haemoglobin determinations on comparable groups of healthy men engaged in sedentary and active pursuits throw doubt on the accuracy of this as a generalization. One of us (Magee, 1946) in commenting on the results of the M.R.C. survey (Committee on Haemoglobin Surveys, 1945) drew attention to the relatively low levels given by agricultural workers compared with civil servants.

Tests made on comparable groups of healthy active and sedentary soldiers, police and civilians go to confirm that inactive persons have significantly higher Hb levels than persons engaged in active or very active occupations (see Table).

| Group | No. of subjects | Mean age (years) | Mean Hb (Haldane) (%) | Range | Standard error | Difference* |
|---|---|---|---|---|---|---|
| 1. Scots Guards, London | 60 | 21 | 99·9 | 96–124 | 0·817 | S |
| 2. R.A.M.C., Millbank | 60 | 21 | 103·0 | 97–123 | 0·682 | |
| 3. A.S.P.T., Aldershot | 60 | 25·5 | 112·0 | 98–128 | 0·917 | S |
| 4. R.A.P.C., Aldershot | 60 | 22·0 | 118·0 | 98–137 | 1·085 | |
| 5. Outdoor staff, Stepney | 18 | 39·0 | 110·0 | 91–115 | 2·243 | Just not S |
| 6. Office staff, Stepney | 21 | 40·6 | 115·0 | 94–127 | 1·528 | |
| 7. Metropolitan police, on beat | 50 | 35 | 108·8 | 96–127 | 1·140 | Not S |
| 8. Metropolitan police, office | 50 | 38 | 109·0 | 95–122 | 0·829 | |
| 9. Manchester, Leeds, Oldham and Birmingham police, on beat | 149 | — | 104·9 | 85–125 | 0·477 | S |
| 10. Manchester, Leeds, Oldham and Birmingham police, office | 106 | — | 108·1 | 92–135 | 0·607 | |

* S = significant.

The procedure of blood sampling was the same for comparable active and inactive groups. For instance, the Aldershot groups (3 and 4) were sampled at the same time on different days over a period of about 10 days and the A.S.P.T. men had not done any physical training on the day of sampling. There were differences between pairs of groups greater than between the individual groups constituting the pair, e.g. group 2 (London) is lower by 15% than group 4 (Aldershot) and only 3% higher than its comparable group 1. There is no reason for thinking that the former differences are related to muscular exercise. Nor is it likely that the cause is variation in diet, for the soldiers' rations were substantially the same. Whatever may be the cause of these differences they do not invalidate the general trend of those between the active and inactive groups. Of the five pairs of comparable groups three show statistically significant differences in favour of the inactive groups; one difference in favour of the inactive group just fails to be significant; the values for the other two groups (7 and 8) are identical. Greater blood volume in active people or less efficient Hb in inactive people are conceivable reasons for the lower Hb in active persons. Dr Hammond of Cambridge is collaborating with us in further inquiries into these matters.

The Hb determinations were made by the alkaline haematin method using the photometer described by King (1947). Frequent checks were also made by the photoelectric method, also described by King (1942). The artificial standard solution was the same as that described by Gibson & Harrison (1945). Both instruments and examples of findings were demonstrated.

REFERENCES

Gibson, Q. H. & Harrison, D. C. (1945). *Biochem. J.* **39**, 490.
King, E. J. (1942). *Lancet*, **242**, 511.
King, E. J. (1947). *Biochem. J.* **41**, xxxii.
Magee, H. E. (1946). *Brit. med. J.* i, 475.
Committee on Haemoglobin Surveys (1945). *Spec. Rep. Ser. med. Res. Coun., Lond.*, no. 252.

**The Absorption and Determination of Helenien in Blood.** By J. G. D. PRATT and MARGARET I. STERN, *Royal Aircraft Establishment and R.A.F. Institute of Aviation Medicine, Farnborough, Hants*

Helenien (lutein dipalmitic ester) has been claimed by von Studnitz (1945) and others to exert a favourable effect on night and colour vision. We have been unable to demonstrate any favourable effects. Helenien was extracted from the petals of French marigolds (*Tagetes patula–flore pleno*), and the resultant red waxy solid obtained in a yield of less than 1%. Four subjects were fed each day for 14 days with doses ranging from 100 to 400 mg. of the ester, which was dissolved in olive oil and incorporated in an emulsion. Blood was drawn at least four times from the subjects and controls, including twice during the actual medication period. The serum, after saponification and extraction (Gillam & el Ridi, 1935), was examined for the presence of the xanthophyll as distinct from $\beta$-carotene, etc. (Karrer, 1945). The results showed that absorption of the xanthophyll and circulation in the blood took place in all the subjects. By the partition method of Clausen & McCoord (1936) it was found, in a further experiment, that the xanthophyll present in the circulating blood after ingestion appeared as an ester; possibly as the original lutein dipalmitate.

REFERENCES

Clausen, S. W. & McCoord, A. B. (1936). *J. biol. Chem.* **113**, 89.
Gillam, A. E. & el Ridi, M. S. (1935). *Biochem. J.* **29**, 2465.
Karrer, P. (1945). *Vjschr. naturf. Ges. Zürich*, **90**, 1.
Studnitz, G. von (1945). Unpublished communications.

**Pyridoxin and Riboflavin Deficiencies in Rats on Synthetic Diets Containing Succinylsulphathiazole.** By K. J. CARPENTER, L. J. HARRIS and E. KODICEK, *Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council*

Rats weighing 55–60 g. were given a synthetic, low-protein diet (containing casein 10·5, cystine 0·15, sucrose 81·35, cotton-seed oil 3 and salt mixture 5%) together with supplements of crystalline vitamins (including pantothenic acid, biotin, folic acid) but no pyridoxin. Growth was depressed compared with the controls receiving pyridoxin, but even after 120 days no deaths had occurred, and no skin lesions were observed. In view of the findings of Cerecedo & Foy (1942) a second group of rats was placed on the same experimental diet, but at the age of weaning. Their condition, however, was not seriously worse than that of the first group. A third group of rats,

on the other hand, receiving this same diet but with the addition of 1% succinylsulphathiazole, began to lose weight after about 34 days, developed severe acrodynia, and died after 45–60 days if left untreated. No convulsions or paralysis were observed. Pyridoxin cured the deficiency. These observations suggest that the delayed appearance of deficiency symptoms on our low-protein diet without pyridoxin may have been due to microsynthesis of the vitamin in the intestine rather than to the utilization of body stores.

On the same diet unsupplemented with riboflavin, but containing pyridoxin, cessation of growth occurred within a week even when no succinylsulphathiazole was given. However, the appearance of rats receiving the drug was worse, and deaths occurred earlier if no treatment was given.

REFERENCE

Cerecedo, L. R. & Foy, J. R. (1942). *J. Nutrit.* **24**, 93.

**Diet and Resistance to Tuberculosis.** By S. R. SENGUPTA, *Rowett Research Institute, Bucksburn, Aberdeenshire*

Work already published (Long, 1941*a*, *b*, 1947; Leitch, 1945; Keers, 1943, 1948; Day, 1942, 1948; Rich, 1946; Dubos, 1947) furnishes sufficient evidence to warrant experimental studies of the influence of diet on resistance to tuberculosis.

Since April 1947, I have been making observations at the Rowett Research Institute on White Swiss mice inoculated intravenously with Martin's (1946) H905 strain of human tubercle bacilli. The mouse is a particularly useful animal for this type of investigation, as Martin and Dubos have both demonstrated. As a measure of resistance I have used the survival time and survival rate of adequate groups of animals and the extent and character of the lung lesions in animals killed both at the end of experiments and at predetermined times after infection. By all these criteria, mice fed on the Rowett Institute stock cube with 14% dried skimmed milk showed higher resistance to experimental tuberculosis than mice fed on a diet containing whole ground wheat 66, whole dried milk 33, and salt 1%. On both diets the mice grew well and reproduced satisfactorily. Attempts will now be made to identify the factor responsible for the increased resistance.

REFERENCES

Day, G. (1942). *Tubercle, Lond.*, **23**, 215.
Day, G. (1948). *Brit. med. J.* i, 363.
Dubos, R. J. (1947). *Experientia*, **3**, 45.
Keers, R. Y. (1943). *Tubercle, Lond.*, **24**, 137.
Keers, R. Y. (1948). *Brit. med. J.* i, 245.
Leitch, I. (1945). *Proc. Nutr. Soc.* **3**, 156.
Long, E. R. (1941*a*). *Arch. Path. Lab. Med.* **32**, 122.
Long, E. R. (1941*b*). *Arch. Path. Lab. Med.* **32**, 286.
Long, E. R. (1947). *Proc. 4th Int. Congr. Microbiol., Copenhagen.* (In the Press.)
Martin, A. R. (1946). *J. Path. Bact.* **42**, 580.
Rich, A. R. (1946). *The Pathogenesis of Tuberculosis*, 1st ed., 2nd printing. Springfield, Illinois: Thomas.

**The Blood Picture in Pyridoxin and Riboflavin Deficiencies in Rats.** By K. J. CARPENTER and E. KODICEK, *Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council*

Blood examinations were made after 30, 70 and 100 days in normal and pyridoxin-deficient rats (see previous communication by Carpenter, Harris & Kodicek, 1948). The deficient animals were found to have a microcytosis—the mean corpuscular volume being 43 as compared with 53 cu.$\mu$. in the controls. The Price-Jones curves also showed a reduction in the mean corpuscular diam. from 7·5 to 6·75$\mu$., but no anisocytosis. However, no animals were anaemic. The mean red blood corpuscle count increased from 7·2 millions/cu.mm. in controls to 8·25 millions in the deficient animals. Large numbers of normoblasts were found in the blood of severely deficient animals. The values for the haemoglobin and 'mean corpuscular haemoglobin concentration' did not change. The total number of leucocytes was not altered but differential counts showed a high proportion of granulocytes (up to 75%). The deficient animals, therefore, showed both granulocytosis and lymphopenia. These results are compared with previous findings in the rat, dog and pig (Cartwright, 1947).

Riboflavin-deficient animals showed no abnormalities in the red-cell picture, but granulocytosis and lymphopenia were again observed.

The blood picture of rats with food intake restricted but fully supplemented with vitamins showed none of the changes found in either pyridoxin or riboflavin deficiency.

REFERENCES

Carpenter, K. J., Harris, L. J. & Kodicek, E. (1948). *Brit. J. Nutrit.* **2**, vii.
Cartwright, G. E. (1947). *Blood*, **2**, 111.

**Preliminary Observations on the Amount of Pteroylglutamic Acid ('Folic Acid') Required by the Rat.** By SYLVIA J. DARKE and C. WHITE, *Physiology Department, University of Birmingham*

A purified diet containing 1% succinylsulphathiazole was fed to weanling rats, and subcutaneous injections of pteroylglutamic acid were given concurrently twice a week. When the intake of pteroylglutamic acid was inadequate the characteristic signs of 'folic acid deficiency' appeared: decreased growth, roughening of the fur, porphyrin staining, and granulocytopenia. If the deficiency became more severe the animals developed alopecia, lymphocytopenia and, occasionally, ulceration of the mouth accompanied by bacteraemia. Anaemia did not usually occur. If the experiments lasted longer than about 4 months some of the animals developed renal calculi from absorption of the succinylsulphathiazole, so that the rat is not a suitable animal for studies of this duration.

For the purpose of this investigation, granulocytopenia was used as an index of deficiency, and an animal was arbitrarily classified as deficient if the granulocytes fell below 500/cu.mm. Twenty-two animals were used, but some were treated with more

than one level of folic acid, so that there were fifty-nine tests in all. The results are shown in the table.

*Adequacy of various doses of pteroylglutamic acid*

| Dose of pteroylglutamic acid/week ($\mu$g.) | No. of animals tested | No. of animals that developed deficiency |
|---|---|---|
| 0·5 | 6 | 6 |
| 2·0 | 17 | 14 |
| 4·0 | 4 | 3 |
| 8·0 | 18 | 5 |
| 16·0 | 7 | 2 |
| 32·0 | 7 | 0 |

The median effective dose was therefore approximately 5·7$\mu$g.

In these experiments with rats there was no evidence to support the clinical finding in human patients that the dose of pteroylglutamic acid has to be increased progressively in order to prevent relapse.