The effects of maternal folic acid and vitamin C nutrition in early pregnancy on reproductive performance in the guinea-pig

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1. The effect of different intakes of folic acid (FA) and vitamin C on pregnancy in the Dunkin–Hartley guinea-pig was examined. Female guinea-pigs were subjected to three graded intakes of FA and vitamin C ('deficient', 'intermediate' similar to recommended daily intakes (RDI), and 'supplemented') during early gestation and up to the time of neural tube closure (17th day of gestation), and then returned to the RDI of these vitamins.

2. Plasma and blood cell concentrations of these vitamins were measured once before and at the end of the dietary treatments. Reproductive performance was assessed in terms of the number of resorbed and aborted embryos and weight and size of the live fetuses on the 36th day of gestation.

3. The short-term deficiency of either of these two vitamins, insufficient to affect maternal health, had a dramatic effect on the reproductive performance.

4. The RDI of FA was significantly less effective than the supplemented intake in preventing embryonic deaths. The RDI of vitamin C produced lighter and smaller live fetuses than the supplemented intake.

5. The implications of these findings with regard to vitamin status in early pregnancy in man are discussed.

Animal and human studies of maternal nutrition during pregnancy have indicated an important role for vitamins in the maintenance of normal gestation and fetal development (Kalter & Warkany, 1959; Hibbard, 1975; Smithells *et al.* 1976, 1980).

Experimental work has indicated that gross deficiences of single vitamins are capable of producing malformations in animal fetuses (Kalter & Warkany, 1959; Warkany, 1965). It is, however, uncommon for man, and indeed animals, to experience such severe degrees of single nutritional deficiency (Giroud, 1959; Smithells *et al.* 1976). Of more relevance are marginal deficiencies of several nutrients which may be detrimental in pregnancy (Smithells *et al.* 1976, 1983), because such deficiencies are encountered in some communities within Western industrial societies (Morse *et al.* 1975; Weighley, 1975; Schorah *et al.* 1978). Little animal experimental work has been undertaken to test the effects of multiple and marginal vitamin deficiences on pregnancy.

While the potential breadth of such a study is enormous, the reported effects of deficiencies of folic acid (FA) and vitamin C on pregnancy outcome (e.g. Nelson, 1960; Stempak, 1965; Hibbard, 1975; Smithells *et al.* 1976; Schorah *et al.* 1978), and the metabolic relation of these two vitamins (May *et al.* 1951; Stokes *et al.* 1975), suggest that a study of the related effects of marginal deficiences of FA and vitamin C might prove the most informative.

Following a preliminary experiment to assess the effect of dietary FA deficiency on the health of adult guinea-pigs, maternal guinea-pigs were subjected to marginal dietary deficiences or supplementations (four to five times the recommended daily intakes (RDI, Navia & Hunt 1976)) of FA and vitamin C (singly or combined) during early gestation and up to the time of neural tube closure. The effects of these dietary treatments on pregnancy were assessed by measuring the rate of fetal death and fetal size and weight in mid-gestation.

MATERIALS AND METHODS

Animal model

The guinea-pig was chosen as the experimental animal because of its dependency on dietary vitamin C (Chatterjee *et al.* 1975), and the similarities of its reproductive physiology to that of the human (Reed & Hounslow, 1971). The outbred Dunkin–Hartley strain (Dunkin *et al.* 1930) was preferred to the inbred strains for its better fertility (Dunkin *et al.* 1930; Rowlands, 1949; Lovell *et al.* 1972) and for its wider availability in defined size and weight (Festing, 1976).

Animal care

Three-tier batteries of galvanized wire cages were used, and these were kept in optimum conditions for this strain of guinea-pig (Ediger, 1976).

The young females, weighing between 300 and 400 g, were initially caged in groups of four and fed *ad lib.* with a rabbit-pellet (SG1) diet (Short & Gammage, 1959) supplemented with a daily ration of about 100 g fresh cabbage per cage. After a rehabilitation period of 7–10 d, the females were weighed and those with below-average weight gain (mean of 4·4 g/d) (Dunkin *et al.* 1930) were excluded. Those remaining were divided into experimental groups matched according to body-weight distribution and were marked by numbered ear-tags.

Animals were kept two or three per cage and were moved as diet changes required. These movements were necessary because of variation in the times of conception and were carried out in such a way that similar frequencies of movements were made within every experimental group; this necessarily also involved moving those whose diet did not change.

Ten sires were each kept, singly, in permanent cages and the females, when in 'heat' (see below), were introduced to these cages for mating which took place immediately or shortly after the introduction of the female (Draper, 1920). The female was allowed to remain with the sire for up to 24 h or until leucocytes appeared in a vaginal smear (Stockard & Papanicolaou, 1917), during which time both the female and the sire received the female's diet.

Assessment of day '0' pregnancy

The time of fertilization (day '0' of pregnancy) was assumed to be 12 h after the onset of heat (Blandou & Young, 1939), which was assessed by thrice-daily observations of the females' behaviour (Loeb & Lathrop, 1914), the vaginal closure membrane (Elvidge, 1972) and vaginal smears (Selle, 1922).

The success of mating was assessed by the presence of a vaginal plug, sperm in the vaginal smear, or both, as described by Stockard & Papanicolaou (1919). Unsuccessfully mated females, all of whom returned to oestrus 14 d later, were eliminated from the experiment.

Assessment of reproductive performance

Pregnancy was confirmed by plasma progesterone values on the 13th and 19th days of gestation (Challis *et al.* 1971), and by abdominal palpation. The presence of a dark-coloured exudate filling the vagina was indicative of embryonic resorption. This exudate was either visible through the vaginal membrane, or its presence was detected by a vaginal smear.

Pregnancies were terminated on either the 38th or 49th days of gestation in the preliminary experiments, and on the 36th day of gestation in the main experiment. The latter was chosen because it marks the end of hyperplastic growth phase when the fetuses resemble the new-born guinea-pigs (Harman & Prickett, 1932), but before the majority of dead fetuses have completely resorbed. Immediately after the pregnant female was killed, by breaking its neck, the uterine horns were exposed and the live fetuses, which were identified by skin colour and spontaneous movements, were counted. Each of these was then weighed and

Table 1. *Expected and measured folic acid (FA) and vitamin C (C) contents of the 'gel' and SG1 diets used*

(Mean values and standard deviations)

	FA (mg/kg)				C (mg/kg)			
		Measured*				Measured		
	Expected	Mean	SD	n	Expected	Mean	SD	n
'Gel' diets								
FA-deficient	0	0·05	0·02	4	2000	1900	160	4
FA-supplemented	10†	10·6	0·5	5	2000	1900	150	4
SG1 diets								
SG1	1‡	2·6	0·6	11	trace	0	0	6
SG1 + 15 mg C/kg	1‡	2·2	0·1	11	15	13·5	2·7	5
SG1 + 2 g C/kg	1‡	1·5	0·1	11	2000	2000	163	6
SG1 + 2 g C + 10 mg FA/kg	11†	11·7	1·2	12	2000	1950	157	6
Cabbage	0·9§	—	—		400§	—	—	

* Monoglutamate forms following hydrolysis with chick pancreas conjugase.
† Added in the form of pteroylglutamic acid.
‡ Food FA mainly in the form of substituted pteroylpolyglutamic acid.
§ Paul & Southgate (1978).

measured for crown–rump length and head circumference. The dead and resorbing fetuses were also counted.

The number of implantations in a uterine horn was determined by two matching criteria: (1) total number of placental attachment sites of surviving and resorbed fetuses (resorption 'scars') on the inferior mesomeric wall of a uterine horn (Wilson, 1973), and (2) total number of corpora lutea of pregnancy (CLP) (Rowlands, 1956) in the adjacent ovary (Wilson, 1973). However, when all the fetuses were dead, all the CLP had regressed (Rowlands, 1956), but they could be quantified by examining the histological sections of the whole ovary fixed in Bouin's fluid and stained in Ehrlich's H and E stain (Disbery & Rack, 1970).

Throughout the study, only one female, which had been regarded as successfully mated, returned to oestrus without showing any signs of previous implantation. Whether the return to oestrus was a result of a mistake in the assessment of mating-success or failure of either fertilization or implantation was not possible to ascertain. Fortunately, the incident was an isolated one and the case was listed as 'failure of conception'.

Diets

Two types of experimental diets were used: a semi-synthetic 'gel' diet based on that described by Navia & Lopez (1973), and a pelleted diet based on Short & Gammage's (1959) diet no. 1 (SG1).

The 'gel' diet was produced in our laboratories from ingredients obtained from ICN Pharmaceuticals Inc., California. This diet, providing two to ten times the RDI of most vitamins (Navia & Hunt, 1976), lacked only *p*-aminobenzoic acid and contained one of two concentrations of FA: deficient or supplemented (Table 1). The supplemented concentration was to provide two to four times the RDI and the deficient concentration provided only a fraction of the RDI (RDI of FA is 100–200 μg).

The SG1 pelleted diet (obtained from Burnhill and Sons Ltd, Northgate, Cleckheaton),

with the addition of about 20 g cabbage/d per animal, provided nutrient intakes similar to, or just below, the RDI. The basic SG1 diet, therefore, provided vitamin intakes which were lower than those provided by either the supplemented or deficient 'gel' diets except for FA where intake was intermediate between the concentrations found in these two diets (Table 1). In some experimental SG1 diets the concentrations of FA and vitamin C were adjusted to resemble the supplemented levels in the 'gel' diets, by grinding the pellets and mixing with extra amounts as required. This powder was then repelleted to form the fortified SG1 diets.

The diets were fed for varying periconceptional periods in order to achieve 'deficient', 'intermediate' or 'supplemented' reserves of FA and vitamin C during organogenesis, i.e. 14th–19th d of gestation in a 68–72 d gestation (Giroud, 1970; Nishimura & Tanimura, 1976).

Pair-fed controls were not used in the present study because of the difficulty in pairing females with coinciding oestrus cycles and therefore coinciding time of conception. However, a daily monitor of food intake and spillages provided a means by which an alteration in the food intake of any dietary-treatment group would be detected.

The diets were analysed for vitamin C (Roe, 1954) and FA concentrations (Chanarin *et al.* 1972) (Table 1) so that daily intakes of these vitamins could be calculated. The concentration of vitamins in cabbage was obtained from food tables (Paul & Southgate, 1978) (Table 1).

Blood measurements

Blood (2·5 ml) was taken from the lateral metatarsal vein by negative pressure from a vacuum pump (Dolance & Jones, 1975) into tubes containing EDTA before and immediately after the dietary treatments and this was assayed for plasma and erythrocyte FA (Chanarin *et al.* 1972) and plasma and leucocyte vitamin C (an adaptation of the technique of Denson & Bowers, 1961). Blood samples (0·5 ml) were also taken on the 13th and 19th days of gestation and these were used for progesterone determination (Illingworth *et al.* 1970) to aid detection of pregnancy.

Assessment of the health of the mother

The females were weighed at 3-d intervals throughout every experiment, and each was subjected to a full blood count and smear once before the dietary treatment (10–3 d preconception) and once after (17th day of gestation).

Statistical analysis

Measurements with a 'normal' frequency distribution were analysed by a parametric analysis of variance (ANOVA). When significant differences were indicated the data were subjected to Duncan's Multiple Range Test (Duncan, 1955) to determine significance of differences between groups. A two-way ANOVA was used for data with repeated measures.

The non-parametric data (the data on pregnancy outcome) were first subjected to variance analysis with Kruskal–Wallis ANOVA (Kruskal & Wallis, 1952), and then the mean values were compared using the Mann–Whitney U test (Mann & Whitney, 1947).

RESULTS

Preliminary findings

The aim of these investigations was to examine the effects on pregnancy of dietary restrictions which were designed to affect neither the implantation of the fetus (6th day of gestation) nor the health of the mother. However, there is little information in the literature about the effects of dietary FA deficiency on the guinea-pig and its pregnancy. Our preliminary investigations indicate that the consumption of the FA-deficient 'gel' diet

Table 2. *Blood folic acid (FA) on the 17th day of gestation and reproductive outcome on the 49th day*

(Mean values and standard deviations)

Dietary treatment group	No. of conceptions	Blood FA (ng/ml)				Average number per conception							
		Plasma		Erythrocytes		Implantations		Live fetuses		Resorptions and abortions		Resorptions and abortions as % of implantations	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	5	6·8	8·8	151	73	5·0	0·7	4·2	0·8	0·8	0·8	15·3	16·6
FA-deficient	3	0	0	38	26	3·0	1·7	1·3	2·3	1·7	2·1	66·7	57·7

Table 3. *Mean daily intakes of folic acid (FA) and vitamin C from the 3rd day preconception to the 17th day of gestation with the resulting blood concentrations on the 17th day and reproductive performance on the 37th day of gestation*

(Mean values and standard deviations)

Dietary treatment groups	Description of the diets	No. of mothers	Daily intakes				Blood concentration						Average no. per conception					
			FA (µg/d per mother)		Vitamin C (mg/d per mother)		Plasma FA (µg/l)		Erythrocyte FA (µg/l)		Leucocyte vitamin C (µmol/l blood)		Live fetuses		Resorptions and abortions		Resorptions and abortions as % of implantations	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1. Intermediate FA and vitamin C	SG1 + daily ration of cabbage	17	110	9	9·1	1·0	1·6	0·7	99	35	10·2	5·1	2·3	2·4	2·6	2·4	53·4	45·7
2. Supplemented FA and vitamin C	SG1 fortified with FA and vitamin C	15	454	34	86·8	6·4	8·3*	3·5	239*	79	22·7*	10·8	4·3†	1·9	1·1	1·7	20·2†	34·0

* Mean values were significantly greater than the corresponding values of group 1 (one-way ANOVA): $P < 0.01$.
† Mean value was significantly less than the corresponding values of group 1 (Mann-Whitney U test): $P < 0.01$.

(lacking both FA and p-aminobenzoic acid (Table 1)) by females for 25 d, resulted in significant decreases in plasma and erythrocyte FA and in neutrophil count, although there was little change in body-weight gain at this stage. Thereafter the females contracted diarrhoea, lost weight rapidly and became severely ill.

To avoid a direct effect on the health of the mother, the dietary FA deficiency was initially confined to a period of 19 d during early pregnancy (3rd day preconception to the 17th day of gestation) in an experiment on a small number of animals. This was achieved by feeding the 'gel' diet deficient in FA (Table 1) between these days (FA intake of about 2 μg/d), after which the animals were returned to the FA-supplemented 'gel' diet (Table 1, mean FA intake of 455 (SD 97) μg/d) and were killed on the 45th day of gestation. Compared with a control group given the FA-supplemented 'gel' diet throughout pregnancy, the FA-restricted animals had very low plasma and erythrocyte FA levels on the 17th day of gestation, and recorded a high incidence of fetal resorptions and abortions with few live fetuses remaining on the 45th day of gestation (Table 2). These results, however, were not compared statistically because of small numbers. No effects on maternal health in terms of weight gain and haematological change were recorded.

Because of the severe effects on pregnancy and the very low maternal blood FA 'concentrations' observed in this experiment, it was decided that even this degree of FA restriction was too extreme and would not be comparable to FA deficiency normally seen in human and animal pregnancies. It was decided, therefore, to investigate the effects of a less severe restriction of FA on fetal development and to compare the results with the effects of FA and vitamin C supplementation.

Thirty-two female guinea-pigs were provided with SG1 diet and the daily ration of cabbage (20 g/d per animal) supplying intermediate intakes of FA and vitamin C (Table 3), which are at the lower end of the range of RDI for these vitamins (Navia & Hunt, 1976). Fifteen of these then received the SG1 diet fortified with FA and vitamin C, increasing their daily intakes of FA and vitamin C by four- and eightfold, between the 3rd day preconception and the 17th day of gestation. All pregnancies were terminated on the 37th day of gestation.

Compared with the animals on the intermediate intakes, the animals receiving the supplemented diet showed significantly higher blood concentrations of FA and vitamin C and a better reproductive performance in terms of reduced fetal mortality and therefore an increased number of live fetuses on the 37th day of gestation (Table 3).

These findings indicated that the intermediate intakes of FA and vitamin C (Table 3), although well above intakes which will induce symptoms of deficiency, were associated with a less satisfactory reproductive performance than when higher intakes were provided for a limited period in early gestation. This finding was surprising and required confirmation and more detailed examination.

Detailed investigation

Diets including all permutations of FA and vitamin C would have required nine different groups. This size of experiment was beyond the resources and facilities of the department. We already had indications that FA alone had a substantial effect on pregnancy, even in the presence of adequate vitamin C (Table 2). The experiment was therefore designed to investigate if fetal development in animals on intermediate intakes of FA could be modified by changes in dietary vitamin C, or if an optimal FA intake was required to improve reproductive performance. The SG1-based diets (Table 1) were given during early gestation to four groups of animals to produce three levels of vitamin C intakes ('deficient', 'intermediate' and 'supplemented') accompanying an 'intermediate' intake of FA in three groups (Table 4, groups A, B and C), and 'supplemented' intakes of both vitamin C and FA in the fourth group (Table 4, group D).

Table 4. *Details of dietary regimens and periods of dietary treatments*

	Dietary treatment group, intended vitamin status*		Period before conception (d)	Period after conception (d)
Group	FA	Vitamin C	Weaning ↓ . . . // . . 3 . . . 0 . . . . . 5 . . . . . 10 . . . . . 15 . . . . . 20 // . . .	
A	Intermediate	Deficient	←——— SG1 + cabbage ———→ ⊣←SG1⊣← SG1 + 15 mg C/kg ⊣← SG1 + cabbage ___
B	Intermediate	Intermediate	←——————————— SG1 + cabbage throughout ——————————→
C	Intermediate	Supplemented	←SG1 + cabbage →⊣←——— SG1 + 2 g C/kg ———→⊣← SG1 + cabbage ___
D	Supplemented	Supplemented	← SG1 + cabbage →⊣←——— SG1 + 2 g C + 10 mg FA/kg —→⊣← SG1 +___ cabbage

C, vitamin C; FA, folic acid.
* Intended vitamin status between 14th and 17th days of gestation.

An FA-deficient diet was not introduced because of the severe effects of this diet on pregnancy which were observed in the preliminary experiments. The dietary regimens, which are shown in detail in Table 4, were designed to produce their maximum effects by the 17th day of gestation without affecting the time of implantation on the 6th day of gestation or the health of the mother.

For this reason it was necessary to provide a rather complex dietary regimen to produce a deficient intake of vitamin C whilst at the same time avoiding the severe effects of such a diet on the animal (Ingier, 1915; Ginter *et al.* 1968). This was achieved by feeding the SG1 diet without the daily cabbage supplement from the 6th to the 9th day of gestation (an intake of $< 4 \mu g/d$) followed by the SG1 diet fortified with 15 mg vitamin C/kg (an intake of about 0·4 mg/d) from the 9th to the 17th day of gestation (Table 4, group A).

When the females were not provided with either the deficient or the supplemented vitamin intakes during the experimental period as indicated in Table 4, group B, they were maintained on the intermediate intakes of these vitamins which were similar to the RDI. These females represented a control group for comparison with those on deficient or supplemented intakes. The average daily intakes of these vitamins between the 3rd day preconception and the 17th day of gestation in the different treatment groups are shown in Table 5.

Plasma and blood cell concentrations of these vitamins on the 17th day of gestation reflected the levels of intake (Table 6). None of the dietary regimens caused a significant variation in blood cell count, but a vitamin-C-deficient diet accompanying the intermediate FA intake caused a reduction in maternal weight gain (Table 7, group A).

In terms of reproductive performance, the results confirm the findings of the previous experiment showing a decrease in the proportions of resorptions and abortions when supplemented intakes of both FA and vitamin C, compared with intermediate intakes, were provided during early pregnancy (Table 8). This effect seemed to be primarily due to the FA supplement because supplemented intakes of vitamin C alone did not improve reproductive performance (Table 8, group C). However, this experiment does not completely exclude the possibility that the vitamins act synergistically to the benefit of the fetus when provided in the diet to supplemented levels.

Table 5. *Average daily intakes of folic acid (FA) and vitamin C between the 3rd day preconception and the 17th day of gestation by the dietary treatment groups*

(Mean values and standard deviations)

	Dietary treatment group, intended vitamin status*		No. of pregnant mothers	FA (μg/d per mother)		Vitamin C (mg/d per mother)	
Group	FA	Vitamin C		Mean	SD	Mean	SD
A	Intermediate	Deficient	16	99	26	0·35	0·1
B	Intermediate	Intermediate	13	107	13	10·7	1·5
C	Intermediate	Supplemented	11	103	15	80·0	13·3
D	Supplemented	Supplemented	14	477	61	75·0	10·4

* Intended vitamin status between the 14th and 17th days of gestation.

Decreasing vitamin C intake alone to an average intake of 0·4 mg/d further reduced the number of live fetuses compared with the provision of intermediate intakes of vitamin C (Table 8). It must be emphasized, however, that this is the one dietary treatment which appeared to affect maternal health, as indicated by loss of maternal weight, in contrast to the other diets where weight increased steadily. Daily food intakes were not affected by any dietary treatment.

Supplementing with both vitamins together also increased the size of the live fetuses. Here, vitamin C supplementation alone was also effective in increasing the size of live fetuses, although there is a suggestion that this effect was less pronounced than that of increasing intakes of FA and vitamin C together to those of supplemented levels (Table 8).

DISCUSSION

Modifications to the dietary intakes of FA and vitamin C during organogenesis, but not implantation, in the guinea-pig pregnancy have led to significant changes in reproductive performance in terms of number and size of live fetuses in mid-gestation. Whilst severe dietary restrictions would be expected to compromise the outcome of any pregnancy, it is surprising that intakes of FA and vitamin C which have been used as feeding stock for guinea-pigs (Navia & Hunt, 1976) and which have no apparent effect on the health of the adult animals can have such a dramatic effect on pregnancy. The number of live fetuses found at termination of pregnancy in the FA- and vitamin C-supplemented females (range 3·5–4·3) was greater than the published average litter size for this strain of guinea-pig (3·0) (Dunkin *et al.* 1930).

The separate contributions of vitamin C and FA cannot be ascertained from the present study. The results on the effect of varying the vitamin C content of the diet with a constant FA intake lend some support to the idea that FA is important in maintaining the number of live fetuses, whilst vitamin C had an effect on the size of fetuses. However, this observation requires confirmation. Vitamin C deficiency caused further reduction in the number of live fetuses, but this may have been secondary to maternal weight loss.

As the SG1 diet was improved by increasing its vitamin C and FA contents only, it is possible that increasing the concentrations of other micronutrients may further improve pregnancy outcome. However, there is some evidence against this, because FA and vitamin C supplementation alone achieved a reproductive performance equivalent to that seen with the supplemented 'gel' diet used in the preliminary experiments in which all vitamins were provided at well above the RDI (Tables 3 and 8, cf. Table 2).

Table 6. *Blood folic acid (FA) and vitamin C concentrations by the dietary treatment groups*

(Mean values and standard deviations for no. of repeated measurements indicated)

Dietary treatment group, intended vitamin status†		No. of mothers	Plasma FA (µg/l)					Erythrocyte FA (µg/l)				Plasma vitamin C (µmol/l)					Leucocyte vitamin C (µmol/l blood)					
			Before dietary treatment‡			After dietary treatment§		Before dietary treatment‡		After dietary treatment§		Before dietary treatment‡			After dietary treatment§		Before dietary treatment‡			After dietary treatment§	
Group	FA	Vitamin C		n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	n	Mean	SD	Mean	SD	n	Mean	SD	Mean	SD
A	Intermediate	Deficient	16	12	1·7	1·3	1·1	1·3	78	31	42	32	14	14·2	9·6	0·6*	1·1	15	9·6	5·0	3·4*	2·8
B	Intermediate	Intermediate	13	11	1·6	1·1	0·7	0·3	85	61	61	37	13	8·5	4·0	5·7	4·0	13	10·2	4·5	9·1	7·9
C	Intermediate	Supplemented	11	8	1·2	1·5	0·8	0·7	115	42	85	20	11	9·6	3·7	34·6*	27·8	11	6·3	3·4	18·2*	15·9
D	Supplemented	Supplemented	14	13	1·3	1·3	7·1*	2·8	121	29	183*	69	14	11·3	7·4	68·1*	24·4	14	7·4	3·4	26·1*	16·5

* Mean values were significantly different from the values before the dietary treatment (two-way ANOVA): $P < 0.01$.
† Intended vitamin status between the 14th and 17th days of gestation.
‡ Before the 3rd day of preconception.
§ On the 17th day of gestation.

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Table 7. *Body-weights of mothers (g) in the four dietary treatment groups*

(Mean values and standard deviations)

	Dietary treatment group, intended vitamin status†		No. of pregnant mothers	Before dietary treatment‡		After dietary treatment§		At pregnancy termination‖	
Group	Folic acid	Vitamin C		Mean	SD	Mean	SD	Mean	SD
A	Intermediate	Deficient	16	714	42	667*	110	579*	141
B	Intermediate	Intermediate	13	725	52	759	53	769*	107
C	Intermediate	Supplemented	11	676	67	722	56	737*	56
D	Supplemented	Supplemented	14	715	47	769	53	852*	53

* Mean values were significantly different from the corresponding values before dietary treatment (two-way ANOVA + Duncan's multiple range test): $P < 0.01$. Between group differences were not significant.

† Intended vitamin status between the 14th and 17th days of gestation.

‡ On the 3rd day preconception.

§ On the 17th day of gestation.

‖ On the 36th day of gestation. This value is excluding the weight of conceptus (i.e. weight of pregnant mother less weight of the uterine contents).

There appears to be no previous work on the effects of FA and vitamin C dietary manipulation during early pregnancy on reproductive performance in the guinea-pig. Rivers & Devine (1975) reported that a daily vitamin C supplement of up to 100 mg/kg body-weight from weaning in female guinea-pigs did not increase the size of their litters. They also noted that a very low vitamin C intake of 1·5 mg/kg body-weight, which was similar to the deficient vitamin C intake in the present study, did not affect the maintenance of the fetuses. The apparent disagreement with the present findings possibly rests on the fact that the FA concentration of the diet they used (6·5 mg/kg) was much higher than the intermediate level used in the present study (2 mg/kg). Possible support for this lies in the findings of Pye *et al.* (1961) who have reported a significant increase in the number and average size of new born guinea-pigs after increasing the maternal vitamin C intake from 2 to 8 mg/d when dietary FA was much lower than that used by Rivers & Devine (1975).

It is known that prenatal deaths and growth retardations occur more frequently in congenitally abnormal than in normal human offspring. We did not observe congenital malformation in the present study, but it is possible that some of the resorbed fetuses may have been malformed. A search for such a possibility would require microscopic examination of embryos in early gestation.

The overall results emphasize the importance of adequate FA and vitamin C intakes in early pregnancy and suggest that such intakes are well above those which prevent the overt symptoms of deficiency. Although intakes of vitamin C higher than the RDI have been recommended for pregnancy in the guinea-pig (Mannering, 1949), a similar recommendation with respect to FA does not appear in the literature. Whether the supplemented intakes of FA and vitamin C provided in this study are optimal for guinea-pig pregnancy remains to be determined.

Extrapolation from the guinea-pig to the human should only be undertaken with caution. The guinea-pig's FA requirement is much greater, on a weight-for-weight basis, than the human's requirement, as is also the requirement for vitamin C (Leutner, 1981). This could imply a difference in the metabolism of FA in the guinea-pig which makes comparison with the human difficult. However, because the mild FA and vitamin C restrictions which impaired the development of the guinea-pig fetus led to blood concentrations equivalent

Table 8. *Reproductive performance in terms of number of live fetuses and rate of fetal death and conceptus weight on the 36th day of gestation*

(Mean values and standard deviations)

Group	Dietary treatment group, intended vitamin status‡ Folic acid	Vitamin C	No. of conceptions	Average no. per conception Live fetuses Mean	SD	Resorptions and abortions Mean	SD	Resorptions and abortions as % of implantations Mean	SD	No. of live fetuses	Crown–rump length (mm) Mean	SD	Head circumference (mm) Mean	SD	Body-wt (g) Mean	SD	Placental wt (g) Mean	SD
A	Intermediate	Deficient	16	0·6*	1·4	3·9	1·5	87·1	29·9	10	49	4	43	3	5·0	1·0	1·3	0·3
B	Intermediate	Intermediate	13	1·4	1·7	3·2	2·0	68·8	35·7	18	50	3	43	2	4·7	0·7	1·5	0·4
C	Intermediate	Supplemented	11	1·4	2·2	2·9	2·1	68·2	46·2	16	52†	1	45†	1	5·6†	0·5	1·6	0·5
D	Supplemented	Supplemented	14	3·5*	1·7	0·8*	1·6	17·5*	35·9	49	54†	2	46†	2	6·2†	0·5	1·7	0·4

* Mean values were significantly different from the corresponding values for group B animals (Kruskal–Wallis+Mann-Whitney *U* test): *P* < 0·01.
† Mean values were significantly different from the corresponding values for group B animals (one-way ANOVA+Duncan's multiple range test): *P* < 0·01.
‡ Intended vitamin status between the 14th and 17th days of gestation.

to those found during some human pregnancies (Smithells *et al.* 1976; Schorah *et al.* 1983), we must exercise caution in assuming that intakes which are sufficient to prevent human scurvy and macrocytic anaemia will also be adequate for pregnancy. The increased demand for FA in human pregnancy is indicated by the prevalence of macrocytic anaemia in late pregnancy in women not given FA supplements (Hibbard, 1975). We are aware that RDI of vitamins are often increased for pregnancy, but in the UK this recommendation does not apply to the first trimester. The findings of this study along with those suggesting a possible reduction in the prevalence of neural tube defect with vitamin supplements (Smithells *et al.* 1980) suggest that the practice of recommending better vitamin intake in late pregnancy (Anderson, 1972; Steingold, 1977) could be extended with benefit to the periconceptional period and early pregnancy.

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