SUMMARY

1. The empirical evaluation of sampling errors of all kinds has in the past been greatly neglected owing to the laborious calculations required on desk machines. With the development of electronic methods these computations can now be readily undertaken.

2. The paper describes a method of evaluating the sampling errors of a numerical sequence using the electronic computer installed at Rothamsted Experimental Station. The method was developed to deal with the data of a dietary survey, but the programme has been written in general form and can take account of missing values.

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The role of fat in the diet of rats

8. Influence on growth of shortening products, 'emulsifier PT 006' and polymerized linseed oil

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In a previous study (Aaes-Jørgensen, 1954) polymerized herring oil given as the sole fat in the diet of newly weaned male rats was found to be toxic. At the level of 7% it depressed growth. At a 28% level the animals were dying after 14 weeks of feeding. Diarrhoea was not a striking sign. In a subsequent experiment a partial polyglycerol ester of in vacuo polymerized soya-bean oil, PT 006 (1 and 3·5% of the diets) was mixed with lard and fed to newly weaned rats in synthetic diets containing 28% lard plus emulsifier. The growth of these animals was almost the same as that of the controls given 28% lard throughout an experimental period of 15 weeks. At the end of this period the animals were killed. At autopsy no signs of carcinomatous tissue were found in the digestive tract.

The present experiments were carried out to study the effect on young rats of diets containing polymerized oils, in particular the effect of a polymerized oil used as an emulsifier in a shortening*, and of the emulsifier itself as the predominant dietary fat component.

* Shortenings (or compounds) are fats used to make pastry, cakes and such-like short, i.e. breaking or crumbling readily.

EXPERIMENTAL

Plan of the experiment. The experiments reported here were divided into two sections, A and B. The composition of the basal diets is shown in Table 1 and of the complete diets in Table 2.

Table 1. *Composition of the basal diets*

	Section A	Section B
Crude casein*	20	20
Sucrose	54	44
Salt mixture†	5	5
Vitamin mixture‡	0·5	0·5
Choline chloride	0·5	0·5

* From Dansk Mejeri Industri & Export Kompagni Ltd, Stege, Denmark.

† McCollum's salt mixture no. 185, supplemented with 13·5 mg KI, 139 mg $CuSO_4.5H_2O$ and 556 mg $MnSO_4.4H_2O$/100 g.

‡ 0·5 g of the mixture consisted of: biotin 0·05 mg, folic acid 0·05 mg, *p*-aminobenzoic acid 35 mg, thiamine hydrochloride 5 mg, riboflavin 5 mg, pyridoxin hydrochloride 5 mg, calcium pantothenate 5 mg, nicotinic acid 8 mg, inositol 15 mg, ascorbic acid 5 mg, DL-α-tocopheryl acetate (Ephynal, Roche Products Ltd) 5 mg, dicalcium salt of 2-methyl-1 : 4-naphthohydroquinone diphosphoric acid ester (Synkavit, Roche Products Ltd) 1 mg, and sucrose to 500 mg.

In section A we have studied the effect on newly weaned male rats of feeding shortening products with the usual content (i.e. about 0·5%) or with ten times that amount of the emulsifier PT 006.

Further, in section B, we have compared the nutritive value of the emulsifier PT 006 itself, and of PT 006 plus a fraction (about 10%) that distils during the polymerization procedure, with the effect of linseed oil polymerized at 275° for 12 h in a strong current of carbon dioxide, as described by Crampton, Common, Farmer, Berryhill & Wiseblatt (1951). In our experiments the polymerized oils were mixed with soya-bean oil and incorporated in the diets.

Section A (groups 143–146) included animals fed on diets with shortening products containing the emulsifier PT 006 or PT 006 plus the product that distils during polymerization in vacuo (see Table 2). Each group consisted of nine animals.

Section B (groups 147–150) comprised animals given PT 006, PT 006 plus distillate, soya-bean oil or polymerized linseed oil. The polymerized oils were always mixed with soya-bean oil in the ratio 2:1 for two reasons: (1) to obtain easy mixing of the fat with the other dietary components, and (2) to obtain diets rich in essential fatty acids. In this section each group was of six animals only.

Animals and their treatment. Newly weaned male rats were used in all experiments. The diets and water were given *ad lib.* for 36 weeks. Vitamins A and D were given as a transparent aqueous colloidal solution, supplying 130 i.u. vitamin A and 20 i.u. vitamin D_2/animal/week. The animals were weighed and inspected weekly and killed with chloroform at the end of the experiment. Autopsies were performed immediately after the animals had been killed. A general autopsy was performed to study the presence of carcinomatous tissue. The kidneys and part of the liver and of the small

intestine were taken for histological examination. The tissues were fixed in 4% formaldehyde, embedded in paraffin, cut in sections of 5 μ, and stained with haematoxylin-eosin. Frozen sections were made of part of the material, which were then

Table 2. *Composition of the complete diets*

Section	Group no.	Type of shortening product* or fat	Amount in diet (parts)	Basal diet A† (parts)	Basal diet B† (parts)	Content‡ in complete diet of: Linoleic acid (%)	Content‡ in complete diet of: Linolenic acid (%)
A	143	Shortening IV§, containing 0·5% PT 006	33·4‖	80	—	2·5	0·5
	144	Shortening V§, containing 5·0% PT 006	33·4‖	80	—	2·2	0·4
	145	Shortening VI§, containing 0·5% (90% PT 006 + 10% PT 006 distillate)	33·4‖	80	—	2·3	0·5
	146	Shortening VII§, containing 5·0% (90% PT 006 + 10% PT 006 distillate)	33·4‖	80	—	2·3	0·4
B	147	PT 006§–soya-bean oil (2 : 1)	30	—	70	5·8	1·1
	148	90% PT 006 + 10% PT 006 distillate–soya-bean oil (2 : 1)	30	—	70	6·5	1·1
	149	Soya-bean oil	30	—	70	16·4	2·8
	150	Polymerized linseed oil§–soya-bean oil (2 : 1)	30	—	70	4·6	4·6

* Percentage composition of the fat mixture of the shortening products:

Coconut oil	40
Hydrogenated coconut oil (m.p. 35°)	20
Hydrogenated whale oil (m.p. 40–42°)	15
Rapeseed oil	25

Percentage composition of the shortening products:

Fat mixture	54·7
Emulsifier or soya-bean oil or both	5·0
Margarine colour	0·3
Water	40·0

† For composition of basal diets see Table 1.
‡ Determined by alkali-isomerization (Stillman, 1949).
§ From Grindstedvaerket A/S, Viby, Jylland, Denmark.
‖ Corresponding to a fat content of 20%.

stained with Sudan III for a study of the fat content. von Kossa's silver-nitrate method was used to demonstrate calcareous deposits. Organs from the animals found dead during the experimental period were not examined histologically.

RESULTS AND DISCUSSION

Growth and morbidity

The average weight increase of the animals in section A (Table 3) at the end of the experiment was about the same as that of the animals given 30% soya-bean oil in the diet (section B, group 149) (Table 4). Table 2 shows that the contents of linoleic acid

and of linolenic acid in the various diets used in section A were almost equal, as determined by the alkali-isomerization procedure (Stillman, 1949).

During the experiment there was a period (from about the 22nd to the 25th week) of colds and pneumonia among the animals. We thought that most animals would survive this period; therefore the experiments were continued, because a long experimental period was desired to study any ultimate carcinogenic effect of the dietary fat. As will be seen from Table 3, the relative growth rates of the different groups of animals in section A were of the same order after the 19th and 36th weeks. No signs of carcinomatous tissue were found in any of the animals in this section.

Table 3. *Mean weights of the groups of rats throughout the experiment, and other information about the rats in section A (nine rats/group)*

Group no.	143	144	145	146
Diet characteristics ...	Shortening IV, containing 0·5 % PT 006	Shortening V, containing 5·0 % PT 006	Shortening VI, containing 0·5 % (PT 006 + distillate)	Shortening VII, containing 5·0 % (PT 006 + distillate)
Weight (g):				
Initial	34·3	34·9	34·8	34·3
After 19 weeks*	334·1 ± 7·4	307·3 ± 7·5	333·0 ± 8·7	312·3 ± 14·2
After 36 weeks*	376·0 ± 10·3	357·7 ± 6·5	369·9 ± 15·2	363·7 ± 18·9
No. of rats alive after 36 weeks	6†	7‡	7§	7‖
Ether-extractable fat in faeces (%)	61·4	55·6	67·5	43·2

* Mean value with its standard error.
† Three animals died from pneumonia after 25, 25 and 27 weeks.
‡ One animal died from gastro-enteritis after 1 week and one died from constipation after 2 weeks.
§ Two animals died from pneumonia after 1 and 24 weeks.
‖ One animal died from pneumonia after 22 weeks and one died after 16 weeks, the cause of death being uncertain.

Average growth and gross signs of the animals in section B are shown in Table 4.

From Table 4 it is seen that all animals in group 150, which were given a mixture of polymerized linseed oil and soya-bean oil (ratio 2:1), died during the first half of the experimental period. Further, these animals grew very slowly. The cause of death was not recorded, because the animals showed cannibalism, so that the carcasses were partly consumed shortly after death. Diarrhoea was moderate and not persistent. This finding corresponds to our experience of polymerized herring oil (Aaes-Jørgensen, 1954). The cause of the poor thriving of these animals is, according to Crampton *et al.* (1951) and Crampton, Common, Farmer, Wells & Crawford (1953), a toxic effect and a lowered digestibility of components of the polymerized fat. Crampton *et al.* (1951) stated that the deleterious effects could be aggravated because of an insufficient supply of essential fatty acids. In the present studies, at least, the dietary amount of these acids was abundant and much higher in section B than in section A.

The growth of the animals fed on the diet with PT 006 mixed with soya-bean oil (ratio 2:1) (group 147), and of those fed on PT 006 plus distillate–soya-bean oil (ratio 2:1) (group 148), was significantly slower than that of the control animals given soya-bean oil (group 149). Of the two groups given PT 006, group 148 grew

considerably more than group 147. This result was somewhat surprising, since it could be expected that the pungent odour of the distillate would have lessened the appetite of the animals. In groups 147 and 148 diarrhoea was found throughout the whole experimental period, somewhat severer in group 147 than in group 148 (Table 4). This may partly explain the poorer growth of the former group. Moreover, the distillate contains a fairly high amount of lower fatty acids, which are easily digested and absorbed, and therefore may have improved growth in group 148.

Table 4. *Mean weights of the groups of rats throughout the experiment, and other information about the rats in section B (six rats/group)*

Group no. ...	147	148	149	150
Type of dietary fat ...	PT 006–soya-bean oil (2 : 1)	90 % PT 006 + 10 % PT 006 distillate–soya-bean oil (2 : 1)	Soya-bean oil	Polymerized linseed oil–soya-bean oil (2 : 1)
Weight (g):				
Initial	34·2	34·3	34·3	33·8
After 6 weeks	79·2	89·2	164·0	59·6 (five animals only)
After 19 weeks*	120·3 ± 4·7	159·5 ± 11·9	308·5 ± 15·0	—
After 36 weeks*	157·2 ± 7·6	201·7 ± 16·6	352·2 ± 21·4	—
No. of rats alive after 36 weeks	5	6	6	0
Weeks on experiment before death	33	—	—	3, 6, 8, 9, 12, 15
Cause of death	Enteritis	—	—	Uncertain
Description of faeces	Dark-green, sticky; severe diarrhoea (1st–36th week)	Dark-green, sticky; moderate diarrhoea (1st–36th week)	Normal	Dark, sticky; moderate diarrhoea
Description of fur	Greasy, soiled with faeces	Yellow-red coloured, especially along the back; in a few instances greasy	Normal	Greasy and thin

* Mean value with its standard error.

As shown in Table 2, the linoleic-acid and linolenic-acid contents of the diets fed to the animals in section B were very high. Comparison of the growth of the animals in groups 147 and 148 with that of group 150 showed that the animals given PT 006 grew slowly throughout the whole experimental period. In these groups (147 and 148) all animals except one were alive at the end of the experiment, whereas all the animals fed on the diet with linseed oil polymerized in a current of carbon dioxide (group 150) were dead after 15 weeks of experiment. The growth of these latter animals was very poor (Table 4).

The explanation of these findings may be found partly in the fact that PT 006 is more hydrophilic than the polymerized linseed oil, i.e. the diets of groups 147 and 148 were possibly more easily digested and absorbed than that of group 150. Further, these results indicate that the nutritive value of a polymerized oil may depend on the oil chosen and on the polymerization procedure. Doubtless polymerization in vacuo and polymerization in a current of carbon dioxide of the same oil will result in products of different chemical composition and maybe of different nutritive properties. Further studies of these matters are in progress.

Autopsy and histology

General. No changes were found at autopsy of the animals in section A (groups 143–146) fed on diets containing shortening products.

Of the rats in section B (groups 147–150) those in groups 147 and 148 reared on diets with PT 006 or PT 006 plus distillate exhibited severe degrees of poor nutrition (cf. Table 4). Further, the mucous membranes of the small intestines were swollen, pale and covered with a thick, slimy exudate. The lymph glands of the mesentery were moderately enlarged. Except for these findings, there were no gross changes.

A summary of the histopathological findings is presented in Table 5.

Table 5. *Histopathological changes in the rats in sections A and B*

			Kidneys*					
Section	Group no.	Diet characteristics	Calculi	Hyaline casts	Degeneration of tubules	Necrosis of papilla	Liver†	Intestine‡
A	143	Shortening IV, containing 0·5 % PT 006	+	±	–	–	f. ±	–
	144	Shortening V, containing 5 % PT 006	±	±	±	–	f. –	–
	145	Shortening VI, containing 0·5 % (PT 006 + distillate)	+	±	±	–	f. –	–
	146	Shortening VII, containing 5% (PT 006 + distillate)	+ +	+	±	–	f. –	–
B	147	PT 006–soya-bean oil	+ + +	+	+ + +	+	c.d., f. –	ent.
	148	PT 006 + distillate–soya-bean oil	+ + +	+ +	+ +	+	c.d., f. –	ent.
	149	Soya-bean oil	±	–	±	–	f. ±	–

* The sign ± indicates traces, +, + + or + + + indicate slight, moderate or marked changes.
† c.d. = degeneration of the central zone of lobules, f. = fatty infiltration.
‡ ent. = chronic hyperplastic enteritis.

Kidneys. In section A, calcified tubules, called calculi in accordance with Borland & Jackson (1931), were present in all groups, but to different degrees. The appearance of the calculi was limited to a zone near the cortico-medullary border, parallel to the kidney surface. The calculi seemed to affect predominantly the ascending limb of Henle's loop. The calculi stained dark blue with haematoxylin and black with von Kossa's stain. They occurred intracellularly in the cytoplasm of the cells of the tubules and were usually so dense that it was impossible to differentiate the nuclei from the cytoplasm. The glomeruli appeared normal in all cases.

In section B calculi were present in abundance in groups 147 and 148. Besides the calcareous deposits near the cortico-medullary border, degeneration of varying degrees occurred in the cortical tubular epithelium. Scattered hyaline casts and a few dilated tubules were present in groups 147 and 148. In these two groups degeneration of the tubules, as characterized by pyknosis and karyolysis of the cell nuclei, was severe. In two rats (one from group 147 and one from group 148) necrosis and calcification of the

renal papilla of one of the kidneys were seen. Part of the necrotic papilla was sloughed off into the pelvis. Pyknosis and karyolysis of the nuclei in the collecting tubules in the kidney papilla of the other rats from groups 147 and 148 were conspicuous, but no calcification had taken place.

Liver. Degenerated liver cells were seen in the central areas of the lobules comprising the inner third of the lobule in all the animals from groups 147 and 148, section B.

Frozen sections stained with Sudan III showed a slight fatty infiltration in groups 143 and 149.

Small intestine. In groups 147 and 148, section B, the surface epithelium was desquamated. The villi intestinales and the mucosa were slightly thickened and infiltrated perivascularly with polyblast-like cells, plasma cells and a few eosinophile leucocytes. Some animals exhibited solitary follicles containing large lymphocytic centres surrounded by densely located lymphocytes.

The histological diagnosis in relation to the macroscopic findings (p. 37) is chronic hyperplastic enteritis.

Borland & Jackson (1931) investigated histologically the kidneys from some of Burr & Burr's (1929, 1930) rats reared on fat-free diets. They found, *inter alia*, papillary necrosis, apical disintegration and calcification, calculi and uncalcified degeneration.

The pathological findings in the kidneys in the present experiments are essentially the same as those described by Borland & Jackson (1931), but calculi at the corticomedullary border cannot, according to our experience, be considered pathognomonic of changes caused by a fat-free diet. In the present experiments all the animals in the various groups were fed on diets rich in essential fatty acids, and, as described above, most animals showed calculi. This finding is in accordance with previous investigations by Aaes-Jørgensen (1953, 1954) and Aaes-Jørgensen, Engel, Funch & Dam (1955), showing calculi on diets with lard, peanut oil, various hydrogenated fats or on fat-free diets. The number of calculi is often highest on the diets richest in essential fatty acids.

SUMMARY

1. Newly weaned male rats were reared for 36 weeks on diets with 30% of various shortening products (supplying 20% fat), or 30% of a mixture of PT 006 (partial polyglycerol ester of in vacuo polymerized soya-bean oil) and soya-bean oil (ratio 2:1), PT 006 plus distillate–soya-bean oil (ratio 2:1), or linseed oil polymerized in a current of carbon dioxide and soya-bean oil (ratio 2:1). A diet with 30% soya-bean oil was used for the control animals. Growth rates were recorded. After 36 weeks a general autopsy was performed, and kidneys, liver and part of the small intestine were taken for histological examination.

2. The animals fed on diets with shortening products grew well and to almost the same extent. Calculi were present in the kidneys to a slight extent only. No pathological changes were found in the liver or small intestine of these animals.

3. The animals reared on a mixture of linseed oil polymerized in a strong current of

carbon dioxide plus soya-bean oil (ratio 2:1) grew poorly, and all died during the first half of the experimental period.

4. The animals fed on a mixture of PT 006 or PT 006 plus distillate–soya-bean oil (ratio 2:1) grew throughout the whole experimental period (36 weeks), although significantly more slowly than the controls. No neoplastic tissue was found at autopsy. Histological examination revealed pathological changes in the kidneys (abundance of calculi, hyaline casts, uncalcified degenerations of the tubules and necrosis of the papilla), liver (degeneration of the central zone of lobules) and the small intestine (hyperplastic chronic enteritis).

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Vitamin B_{12} and protein metabolism

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In the last few years numerous papers have linked vitamin B_{12} in a general way with the metabolism of proteins in different animals.

We reported some years ago (Henry & Kon, 1951) that addition of vitamin B_{12} increased the biological value of casein for the vitamin B_{12}-deficient rat. Indian authors (Marfatia & Sreenivasan, 1951; Baliga & Rajagopalan, 1954; Baliga, Balakrishnan & Rajagopalan, 1954) obtained similar increases for vegetable proteins with normal rats. There was, however, a growing indication of a more specific connexion between vitamin B_{12} and biosynthesis of the essential amino-acid methionine (see, for example, Schaeffer, Salmon & Strength, 1949; Oginsky, 1950) through its effect on the formation of methyl groups (see review by Arnstein, 1955). We have since investigated the bearing of these findings on our original observation, and the results are set out below.