III. THE EFFECT ON KITTENS OF A DIET DEFICIENT IN ANIMAL FAT.

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Mellanby [1919] in the course of his experiments on dogs has come to the conclusion that rickets is a dietetic disease, and that the ultimate cause is a deficiency in the diet of "anti-rachitic factor" a substance which he thinks is probably identical with fat-soluble *A*. He has said that 100 % of young puppies fed on his basal diets develop rickets.

Since the cat, like the dog and man, subsists on a mixed dietary it was decided to investigate whether kittens fed on a diet similar to Mellanby's basal diet would develop rickets. Before describing the experiments on kittens it is of interest to compare the normal diet of young pups and kittens [Tibbles, 1912]:

	Cat's milk	Bitch's milk	Cow's milk
	%	%	%
Protein	9.12	11.15	3.57
Fat	3.33	9.57	3.69
Sugar	4.91	3.08	4.88
Ash	0.61	0.73	0.71

It will be seen from the foregoing table that the percentage of protein is far higher in the milk of the cat and the bitch than in that of the cow; bitch's milk contains nearly three times the percentage of fat present in cat's milk, and the fat content of cat's milk and cow's milk is roughly the same. If the percentage of fat in these three milks could be taken as an index of the quantity of fat-soluble *A* present it would point to the fact that the puppy requires a large quantity of this in its diet, and would afford an explanation on Mellanby's hypothesis of the apparently great liability of young dogs to rickets as compared with other domestic animals.

The latest of Mellanby's published basal diets consists of the following substances:

Separated milk	...	250-350 cc. daily
White bread...	...	<i>ad lib.</i>
Linseed oil	...	5-15 cc. "
Yeast	...	5-15 g. "
Orange juice...	...	3 cc. "
Sodium chloride	...	1-2 g. "

All the fat-soluble *A* content of milk is not present in the fat [see McCollum, 1917], but no accurate data are at present available from which it is possible to make any trustworthy calculation of the amount of this factor present in the quantity of separated milk (250–350 cc.) given in the foregoing diet.

EXPERIMENT 1.

Housing and Diet of Kittens.

The five kittens used in the experiment belonged to three litters. They were housed and fed in two pens (controls in one and experimental animals in the other) in a warmed room. They were allowed out for several hours every day and had abundant exercise. The diet is given in the following table:

Control kittens, Nos. 2 and 3					Experimental kittens, Nos. 1, 4, and 5
Whole milk <i>ad lib.</i>	...	...	...	...	Machine skimmed milk with 3 % olive oil <i>ad lib.</i>
White bread <i>ad lib.</i>	...	...	...	...	Same as for controls
Orange juice 1 cc. (hand fed) alternate days	...	...	...	...	
Marmite 2 g. given in milk, whole not consumed	...	...	...	...	

The milk, whole and skimmed, was brought to the boil and allowed to cool before being given to the kittens—a proceeding which probably destroys only a very small part of its anti-scorbutic value [Barnes and Hume, 1919]. The average fat content of a week's supply of machine skimmed milk estimated by Soxhlet's method was 0.15 %¹. The olive oil used after the first six weeks was kindly supplied by Dr Zilva from a consignment of autoclaved oil which gave a negative result for fat-soluble *A* in rat experiments (linseed oil had been tried but was refused by the kittens). The oil emulsified fairly well with hot skimmed milk but separated out when allowed to stand, so that the quantity of oil consumed was not necessarily 3 % of the skimmed milk intake. The dose of orange juice was a small one, but it was thought that with a diet consisting chiefly of milk a small dose would ensure against anti-scorbutic deficiency. A larger amount was at first given but appeared to give rise to diarrhoea.

The marmite, which was used in place of the yeast to supply additional water-soluble *B* accessory food factor, was given in the day's supply of milk. It is not possible to give the food intake of each individual kitten as the food (with the exception of the orange juice which was given with a pipette) was left in the pens. But the following table gives the average consumption; the quantity of bread is a very rough approximation as it was impossible to estimate with accuracy the residues left.

¹ On a few occasions dried machine skimmed milk or hand skimmed milk was given on account of the failure of the supply of separated milk from the dairy.

Average daily food consumption of each kitten of experiment 1.

	Control kittens 2 and 3	Experimental kittens 1, 4, and 5
Whole milk	About 200 cc. each	—
Skimmed milk with 3 % olive oil	—	About 145 cc. each
White bread	25 g. „	20 g. „
Orange juice	0.5 cc.	0.5 cc.
Marmite	1-2 g.	1-2 g.

Course of experiment.

The accompanying charts (Figs. 1 and 2) show the weight curves of the kittens. But at this stage it must be mentioned that every one of the five kittens, especially the three experimental animals, was latterly very heavily infested with fleas, and that there were in each case at post mortem vast numbers of tapeworms in the small intestines. This tape worm, the *Dipylidium caninum*, has for its secondary host the dog or cat flea. To what extent these parasites affected the ultimate result is not clear, but all the kittens suffered from time to time with loose stools, their rest was disturbed by the fleas, and at the end of the experiment neither of the two control animals was in perfect condition; kitten 2 was well nourished and active but had loose stools, No. 3 was very thin. The experimental kittens were from the first more keenly interested in their food than the controls—they appeared to feel more hungry though their consumption of food was on the average smaller. About the sixth week of the experiment it was obvious that the experimental kittens were thinner than control kitten No. 2 which showed a steady gain in weight after the first three days (control No. 3 was however thin also). By the tenth or eleventh week the experimental animals were extremely emaciated, with coats in poor condition and were becoming less active. The emaciation, the condition of the coat and the inactivity became steadily worse and latterly the abdomens were much distended. At the end of the experiment there was an obvious difference in size between the two control kittens and the other two of the same litters. The difference in length from tip of nose to end of tail was 2 ins. and 1 in. respectively.

All the kittens appeared anaemic, possibly partly as a result of the tape worm infection. The blood count for kitten 5 (on the deficient diet) on the 114th day of the experiment is given below:

	Kitten 5	Normal blood count for cat [Gulland and Goodall, 1914]
Red blood corpuscles	4,390,000 per cmm.	8,000,000 per cmm.
Haemoglobin	21 %	70 %
White blood corpuscles	22,300 per cmm.	18,000 per cmm.

One of the skimmed milk animals, kitten 1, died on the 82nd day, the other two, kittens 4 and 5, were killed on the 114th day.

Post mortem findings.

At post mortem no evidence of rickets was discovered. The pathological findings are given below. It will be remembered that the three kittens on the deficient diet were kittens 1, 4 and 5, those on the theoretically complete diet kittens 2 and 3.

Fig. 1.

Control Kitten, 3 ♀ o—o } Same litter (6 weeks old)
 Experimental Kitten, 4 ♀ ••••• }

It will be seen that this Control Kitten did not thrive

Fig. 2.

Control Kitten, 2 ♀ o—o } Same litter
 Experimental Kitten, 1 ♀ ••••• }
 Experimental Kitten, 5 ♀ x—x }

EXPERIMENTAL KITTENS 4 AND 5. Killed 114th day. Extremely emaciated.

Costo-chondral junctions slightly enlarged. The histological changes as will be seen from the following paper written by Miss F. M. Tozer [1921] resembled those found in guinea-pigs and rats receiving a diet deficient in fat-soluble A.

Stomach and intestines were distended with more or less complete loss of the normal puckering. The walls were much thinner than in the normal animals. The changes were similar to those described by McCarrison [1920] in animals receiving deficient diets. *Liver* pale and fatty looking—that of kitten 5 small compared to the controls. *Kidneys* very pale. *Thymus* absent.

EXPERIMENTAL KITTEN 1. Died 82nd day. Extremely emaciated.

Costo-chondral junctions very slightly enlarged [see Tozer, 1921]. *Stomach and intestines* appeared normal. *Liver* pale fatty and small. *Kidneys* appeared normal. *Thymus* absent.

CONTROL KITTEN 2. General condition excellent, nothing abnormal discovered excepting the parasites which were found in all the animals.

CONTROL KITTEN 3. Very thin. No cause found unless the condition was due to the presence of the parasites.

The teeth of all the kittens were perfect. In the two control animals whose costo-chondral junctions were normal [but see Tozer, 1921] the thymus gland was present, whereas as will be seen above it was absent from the three experimental animals.

Summary. To summarise it appears that the chief effects of the diet deficient in fat-soluble *A* on these three six-week-old kittens were emaciation, arrest of growth, abdominal distention with (in two animals) atrophy of the walls of the stomach and intestines, and changes in the costo-chondral junctions similar to those seen in guinea-pigs and rats suffering from deficiency of the same accessory food factor.

The weak point of the experiment is of course the presence of the intestinal parasites and the fact that one of the two controls did not increase satisfactorily in weight.

EXPERIMENT 2.

Children who develop rickets generally do so during the period of the first dentition—a stage of development at which they are, comparatively, much less mature than six-week-old kittens. Therefore while the first experiment was running it was decided to start a second one, this time with younger animals.

Age and diet of kittens. Of a litter of four kittens aged 16 days, three, after certain preliminary “test feeds,” were removed from the mother and hand fed entirely until nearly five weeks old, and partially until 7–8 weeks old. They were fed three-hourly from 10 a.m. to 10 p.m. with warmed food from a pipette or baby’s bottle until old enough to feed themselves. The food was on the same lines as in the last experiment.

Average daily food consumption of each kitten of experiment 2:

	Control kitten 9	Experimental kitten 6	Experimental kitten 7
Whole milk	About 180 cc.	—	—
Skimmed milk with 3 % olive oil ...	—	About 145 cc.	About 160cc.
White bread supplied <i>ad lib.</i> after 3rd week of experiment	A very small quantity	} Same as for control	
Orange juice hand fed after 3rd week	0.5 cc.		
Marmite	1–2 g.		

Course of experiment (see Fig. 3).

The two experimental kittens appeared always ravenous, though latterly their food intake was decidedly smaller than that of the control. This was a striking feature almost to the time of death. About the 4th day kitten 7 had diarrhoea, a condition from which both experimental kittens frequently suffered, especially kitten 7 (though both had masses of hard faeces in the large intestine at death). By the 8th day kitten 7 was less active than the control, the abdomen was distended and it walked very unsteadily on a broad base with the back bowed. By the 12th day kitten 6 was in much the same condition. They became progressively worse in all respects, the coat was matted and dirty and swarming with fleas, the hair coming out and there

Fig. 3.

Control Kitten, 9   ♀ o—o
 Control Kitten, 8. Left with mother, final weight indicated → x
 Experimental Kitten, 6 ♂ x—x
 Experimental Kitten, 7 ♂ •.....•

was emaciation and arrest of growth. By the end of the experiment the contrast was striking. Kitten 9, though considerably smaller than the single kitten left with the mother, was a healthy active young cat, whereas the two animals on deficient diets were wasted dwarfs with ballooned abdomens and matted hair. They presented a miserable spectacle. The lengths of the kittens quoted below give no idea of the apparent difference in size. Kitten 7 died on the 38th day with an attack of vomiting, kitten 6 appeared moribund and was chloroformed on the 54th day.

Kitten	Comparative lengths of kittens (inches)	
	35th day	54th day
9	14.75	17.75
7	12.5	—
6	13.0	14.75

Post mortem findings.

At post mortem it was found that all three kittens had tape worms, the control far more than the two on the deficient diet. The fat and the healthy looking well-developed muscles of control kitten 9 were in marked contrast to the pale flabby wasted tissues of the two small experimental kittens. The two experimental animals had slightly enlarged costo-chondral junctions (for the histological changes, which resembled those found in the earlier experiment, see Tozer [1921]). The rib junctions of the control were normal. The teeth were perfect.

The *stomach and intestines* showed dilatation and atrophy of the wall. The *liver* was pale and small compared to that of kitten 9. The *kidneys* appeared normal. The *thymus* was absent from all three kittens.

Summary. The results of this experiment were similar to those of the last, but the arrest of growth, emaciation, abdominal distention, weakness and the hankering for food were early and very marked features. The picture seems extremely like that presented by children suffering from coeliac disease, a condition in which there is failure to digest fat, arrest of growth, emaciation, distention of the abdomen and hankering for food, as well as, in some cases, a diminution in size of the liver. Still [1918], after remarking on the apathy and langour of children with coeliac disease, observes "There is one subject in which almost all children with coeliac disease show a pathetic interest—namely their food; often indeed it is their one subject of conversation." He however attributes this to the meagre dietary which has formed a part of their treatment.

Criticisms of the experimental procedure.

If these experiments were repeated it would be desirable to feed the kittens separately in order to ascertain the food intake of each, and for the same reason to give the oil by hand instead of as an emulsion in the milk.

A better control would be provided by feeding the whole litter on skimmed milk, and to the control animals giving say cod liver oil, which is rich in fat-soluble *A*, in place of the olive oil of the experimental animals, for it is probable that the mechanical factor introduced by the oil was in part the cause of the diarrhoea in kittens 6 and 7. It might be as well also to give a larger dose of anti-scorbutic, possibly in the form of neutralised orange juice.

As already pointed out the presence of the tape worms and their hosts the fleas introduced an unlooked-for difficulty.

EXPERIMENT 3.

Young rats aged 13 days (the eyes had opened on the 12th day) were put on a diet of white bread, skimmed milk with 3 % olive oil and small pieces of orange pulp. They consumed a much smaller quantity of milk as compared

with bread than did the kittens—but as will be seen from the weight chart (Fig. 4) their weight was not greatly below normal at the end of 122 days—their general condition was excellent. It would appear therefore that kittens require a larger proportion of fat-soluble *A* in their dietary than do young rats.

Fig. 4.

Control rat, 4 ♀	——	Whole milk, bread and orange juice from 13 days old
„ rat, 7 ♀	----	Left with mother—mixed diet
Experimental rat, 1 ♀	× × — × ×	} Skimmed milk with 3 % olive oil, bread and orange juice from 13 days old
„ rat, 2 ♀	++++	
„ rat, 5 ♀	.....	

GENERAL SUMMARY AND CONCLUSIONS.

In the experiments described above it was found that the six-week-old kittens when fed on a diet deficient in fat-soluble *A*, but otherwise theoretically adequate, became emaciated and finally ceased growing. They suffered from abdominal distention and diarrhoea; at post mortem it was found that the walls of the intestine were thin, and there were changes in the costo-chondral junctions resembling those found in young guinea-pigs and rats suffering from deficiency of the same accessory food factor. The thymus was present in two control animals and absent from the three experimental animals, but in the next experiment was absent from control and experimental animals alike. The food of these experimental kittens consisted of skimmed milk, olive oil and bread, with marmite and orange juice. Some weak points in the experiment have been pointed out.

Analogous changes were observed in a second experiment on kittens aged 16 days, which were hand-fed, and given a similar dietary—but in this case all the changes were much more rapid and striking. The appearance presented by these animals was extremely like the clinical picture of coeliac disease in children. There was no evidence of rickets found in any of the kittens post mortem, but the experiments appear to indicate that kittens when given a

diet deficient in fat-soluble *A* develop a condition similar to that found in guinea-pigs and rats under the same circumstances, and that kittens are probably more sensitive than young rats to a partial deficiency of the fat-soluble accessory food factor.

In conclusion I should like to offer my sincere thanks to Dr C. J. Martin for his stimulating assistance and advice. The greater part of the laborious task of feeding the animals was carried out by Miss H. Henderson Smith, and to her as well as to Miss S. Rutherford and others who shared in the work I owe the possibility of carrying out the experiments.

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