V. COMPARISON OF THE GROWTH-PROMOTING PROPERTIES FOR GUINEA-PIGS OF CERTAIN DIETS, CONSISTING OF NATURAL FOOD-STUFFS.

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INTRODUCTORY.

IN the course of researches upon guinea-pig scurvy, which have extended over several years, evidence has gradually accumulated which seems to show that the guinea-pig, for healthy growth and maintenance, requires an abundant supply of the fat-soluble *A* accessory factor of McCollum (vitamin *A* of Drummond [1920]). Two distinct lines of evidence support this conclusion.

The first line of evidence is derived from a number of experiments in which guinea-pigs grew on one group of diets and failed to grow on another group of diets. The distribution of vitamin *A* among foodstuffs has hitherto been only partially mapped out by the various workers in that field, yet, as far as the value for that factor of the various foodstuffs used is known, those which promoted growth seem to agree in containing vitamin *A* and those which did not, in lacking it. Furthermore those foodstuffs which produced partial growth seem to have done so in proportion to their known relative value for vitamin *A*. It is true that some of the diets which did not promote growth were open to criticism in some other respects than a deficiency of vitamin *A*, but it is also true that some diets on which growth took place were equally liable to have been deficient in the same particulars and yet promoted growth. The only dietary common factor, absent from the one set of diets and present in the other set, was vitamin *A*.

Most of the diets to be described were planned for the purpose of investigating the anti-scorbutic value of certain foodstuffs and in the very earliest experiments the need for vitamin *A* to be included in the basal diet was not recognised. It was, however, soon found that on many diets the animals did not thrive [see Chick and Hume, 1917], although they did not develop scurvy, and from this time onwards 60 cc. daily of milk (autoclaved at 120° for an hour, to destroy the anti-scorbutic factor present) was made a routine addition to most experimental scurvy diets; later on, as the result of added experience, the daily ration was often increased to 90 cc. for large and quickly growing guinea-pigs.

The second line of evidence is that derived from the histological examination of the rib junctions of all the experimental guinea-pigs, which was carried out as a routine by Miss F. M. Tozer. In the course of this examination she was forced to the conclusion that a bone lesion can occur on certain diets in which there can be no possible suspicion that the anti-scorbutic ration is insufficient. This bone lesion is in some stages indistinguishable from the bone lesion of scurvy and it occurs on many different diets which however have in common a deficiency in vitamin *A*. It is described by Miss Tozer [Delf and Tozer, 1918; Tozer, 1921]. The second line of evidence agrees with and supports the first in that the bone lesion is most severe where growth is least and in that failure in growth and severity of bone lesion both appear to be inversely proportional to the amount of vitamin *A* in the diet.

It is only proposed here to bring forward the facts which form the first line of evidence, *i.e.* to set out and compare the growth occurring in guinea-pigs on certain diets, differing from one another in their content of vitamin *A*. Miss Tozer's histological diagnosis of each case, wherever such was made, will at the same time be given for comparison, by her kind permission, but a full histological treatment of the whole question will be made by her separately in another communication.

Reference to the probable need of the guinea-pig for vitamin *A* has frequently been made in the papers of Dr Harriette Chick and her co-workers at the Lister Institute [Chick, Hume and Skelton, 1918; Delf and Skelton, 1918; Chick and Campbell, 1919]. Frölich [1912] refers to a fragility of the bones, attributed to a deficient diet, which is not scurvy. Jackson and Moore [1916] speak of a lesion resembling macroscopically a rachitic rosary, produced in guinea-pigs on a diet which was not quantitatively controlled but may have been deficient either in the anti-scorbutic or in vitamin *A* or in both. Hess and Unger [1918] also refer to changes in the rib junctions of guinea-pigs, on experimental diets, which they regard as more akin to rickets than to scurvy. It is thus apparent that a number of observers have noted the occurrence of more than one bone lesion in guinea-pigs, due to dietary causes, but there does not seem to be much evidence correlating any such lesion with a deficiency of vitamin *A*.

Cohen and Mendel [1918] conclude that the fat-soluble factor is not one of the primary factors concerned in scurvy of the guinea-pig, but do not state whether they consider it may have other importance.

TECHNIQUE.

The technique employed was the same as that which has been used throughout the Lister Institute scurvy experiments and has been detailed many times already. Young growing guinea-pigs about 320 g. weight were fed with a mixture of oats and wheaten bran *ad libitum*, but weighed. To this was added the food to be tested in weighed or measured quantities; the residues were either fed by hand or were measured and the quantity actually consumed

was calculated. In the present set of experiments, extra anti-scorbutic in the form of orange juice was added in those cases where the experimental ration did not already contain sufficient of that factor. It is proposed to give here details of various additions to this basal diet, together with details of the growth on such variously supplemented diets and a histological report, furnished by Miss Tozer.

The comparative growth of the animals has been set out in the form of a table, in which the weight at the start of the experiment and after 25, 35 and 45 days is given, together with the percentage increase for each animal and the average percentage increase for the whole group over each period. The normal as estimated by Delf [Delf and Tozer, 1918] is taken as the standard and in these experiments all degrees of deviation from it are found, down to cases such as Nos. 628, 629, 631, 632 (Table II, Group 28) on a diet of oats, bran, water and 10 cc. of orange juice daily, which showed no growth at all even in the first fortnight, but an actual loss in weight, amounting to an average of 19.7 % for the group for 25 days.

The histological bone lesion occurring in the rib junctions has been classified into the four very convenient degrees of severity already briefly described by Miss Tozer [Delf and Tozer, 1918]. She classifies the lesion occurring in guinea-pigs fed on a diet deficient in the anti-scorbutic or in vitamin *A* into four degrees of severity; normal, incipient, definite, acute; the exact significance of these terms can be ascertained on reference to her paper where diagrams are given. In some cases the experiments were continued for considerably longer than the 45 days for which the latest weight reading is given. In other cases the animal died after as short a period as 20 days so that the histological examination was made after the animals had been for very varying periods on the different diets. Doubtless this variation must affect to some degree the severity of the bone lesion but Miss Tozer has found that histological bone changes begin very early on a deficient diet [Tozer, 1921] and it is not therefore thought that such a variation seriously affects the comparative value of the histological findings, relative to one another. In the last column of the table the number of days elapsing between the beginning of the experiment and the date of death is noted.

Beyond the loss in weight and the bone lesion described, there are no other marked symptoms. The emaciation may be extreme, not a particle of fat being discoverable on the body in severe cases. All bones are more or less brittle and the animal is extremely prone to suffer from intercurrent disease, particularly lung affections.

EXPERIMENTAL RESULTS.

The experimental results can be divided roughly into two groups, those concerned with foods which promoted growth (Table I) and those concerned with foods which did not (Table II). Naturally the division is only an artificial one, for many of the foodstuffs not promoting growth might promote

growth if given in much larger quantities and many of the growth-promoting ones would fail to do so if given in much smaller quantities, but for purposes of convenience such a division is permissible. The first or growth-promoting group includes milk in several forms, green cabbage or some modification thereof and hay. The second or non-growth-promoting group includes white cabbage and a derivative thereof, swede juice, boiled onion, orange juice and germinated peas.

Green cabbage leaf, raw. (Table I, Groups 1, 2 and 3.)

Group 1. 30 g. daily ration. An oat and bran diet to which 30 g. daily of raw green cabbage is added is richly growth-promoting and the average percentage increase for a group of animals for 45 days was 60·4 %, actually more than the normal standard of 55·8 %, calculated for the same period.

Histological examination of the costo-chondral junctions of the ribs. Only two animals were examined, after 39 days of the experiment, and both showed a normal histology.

Group 2. 15 g. daily ration. With a 15 g. daily ration, good growth but not to the full normal extent is induced. The average percentage increase for a group of seven animals for 45 days being 40·1 % which is only about two-thirds of that on 30 g.

Histological examination. Only four animals were examined; three were quite normal and the fourth was nearly so, not being sufficiently abnormal to be classed as "incipient."

Group 3. 5 g. daily ration. A ration of 5 g. daily also produced growth, but the increase was still further below the normal, being an average of only 29·9 % for a group of three animals for 45 days, *i.e.* about half the corresponding figure for 30 g.

Histological examination. The three animals in this group were all examined; one was not quite abnormal enough to be called incipient and the other two were between incipient and definite.

Green cabbage leaf, heated for one hour at 100°. (Table I, Groups 4 and 5.)

The cabbage was prepared by being steamed for an hour at 100°.

Group 4. 30 g. daily ration. On a 30 g. daily ration a group of three animals showed a rate of growth equal to that on 30 g. raw cabbage. The two animals on which the experiment was continued for 45 days showed an even better average rate of increase (71·4 %) than the rate of increase for 30 g. raw cabbage (60·4 %).

Histological examination. All three animals were examined, two of them were found to be perfectly normal and the third showed a very slight departure from normality but not sufficient to be called abnormal.

Group 5. 15 g. daily ration. Three animals received a daily ration of 15 g. and showed a rate of growth (45·5 %) slightly superior to that (40·1 %) for 15 g. of raw cabbage.

Table I.

Group no.	Additions to basal diet of oats, bran and water	Amount of ration	No. of animal	Initial weight g.	Wt after 25 days g.	% increase	Wt after 35 days g.	% increase	Wt after 45 days g.	% increase	Histology			Length of life from beginning of expt. to post-mortem, days
											Normal	Incipient	Definite	Acute
1	Normal	...	...	340	450	32.3	500	47.0	535	55.8				...
	Green cabbage leaf, raw	30 g.	1	285	380	33.3	415	45.6	470	64.9	Not examined			...
	"	"	8	280	365	29.8	415	48.2	475	69.6	"			...
	"	"	351	368	470	27.7	490	33.1	520	41.3	"			...
	"	"	352	338	480	42.0	540	59.7	565	60.5	"			...
	"	"	885	340	440	29.3	480	41.1	510	50.0	"			...
	"	"	150	325	477	46.7	541	66.4	572	76.0	"			...
	"	"	152	315	440	39.6	473	50.1	...	...	"			39
	"	"	153	330	418	26.6	458	38.7	...	...	"			39
	Average	...	...	Average	...	34.2	...	47.8	...	60.4				...
2	Green cabbage leaf, raw	15 g.	359	350	470	34.2	480	37.1	480	37.1	Not examined			...
	"	"	353	345	443	28.4	472	33.9	495	43.4	"			...
	"	"	886	310	340	9.7	370	19.3	400	29.0	"			...
	"	"	826	340	440	29.4	460	35.2	515	51.4	"			...
	"	"	823	370	445	20.2	450	21.6	495	33.7	"			93
	"	"	70	330	435	30.4	485	46.9	510	55.5	"			...
	"	"	71	305	390	27.9	375	22.9	400	31.1	Not examined			71
	"	"	689	321	388	20.8	425	32.4	...	...	"			38
	"	"	693 A	330	348	5.4	...	...	...	...	"			25
	Average	...	...	Average	...	22.9	...	31.1	...	40.1				...
3	Green cabbage leaf, raw	5 g.	421	330	378	14.5	390	18.1	420	27.2	+	?	...	94
	"	"	422	320	340	6.2	362	13.1	403	25.9	+	+	to	94
	"	"	425	318	355	11.6	398	25.1	435	36.7	+	+	to	94
	Average	...	...	Average	...	10.8	...	18.7	...	29.9				...
4	Green cabbage steamed 100°C.	30 g.	333	330	465	40.9	525	59.0	580	75.7	+	to	+	84
	"	"	334	305	395	29.5	455	49.1	510	67.2	+	...	...	84
	1 hr.	"	335	315	392	23.1	435	38.0	...	...	+	...	...	86
	Average	...	...	Average	...	31.2	...	48.7	...	71.4				...
5	Green cabbage steamed 100°C.	15 g.	338	300	385	28.3	440	46.6	465	55.0	+	nearly	...	102
	"	"	339	330	395	19.7	438	32.7	460	39.3	Not examined			...
	"	"	340	350	405	15.7	465	32.8	498	42.2	"			...
	Average	...	...	Average	...	21.2	...	37.3	...	45.5				...

6	Green cabbage steamed 100°C. 2 hrs.	15 g.	CJ 354 355 356 357 358	320 330 330 335 340 350	400 398 413 415 435	25-0 20-6 16-5 22-0 31-8	430 430 442 430 452	34-3 30-3 24-5 26-4 39-9	452 448 470 470 480	38-1 35-7 32-3 38-2 45-4	+	...	Not examined	...	96
7	Raw cabbage juice, filtered ...	20 cc.	CJ 1 CJ 2	338 332	380 350	18-3 5-4	425 380	25-7 14-4	454 380	24-3 14-4	+ nearly	...	...	...	108 109
8	Raw cabbage juice, filtered ...	30 cc.	CJ 10	Average	...	11-8	430	20-0 29-9	440	24-3 32-9	...	+	to	+	100
9	Raw cabbage juice, unfiltered	30 cc.	CJ 9	Average	...	23-8	425	29-9 26-8	440	32-9 31-0	...	+	to	+	100
10	Heated cabbage juice, filtered	20 cc.	CJ 5 CJ 6	Average	...	19-4	316 369	26-8 - 3-9 + 11-1	440	31-0 - 11-2 + 3-9	...	Not examined	...	...	...
11	Heated cabbage juice, filtered + 2 g. casein	20 cc.	CJ 4	Average	...	9-0	303	3-6 - 8-1	342	- 3-6 3-6	...	Not examined	...	...	...
12	Heated cabbage juice, filtered	30 cc.	CJ 3	Average	...	7-6	428	- 8-1 29-6	456	3-6 38-3	...	Not examined	...	...	...
13	Heated cabbage juice, un- filtered	30 cc.	CJ 7 CJ 15	Average	...	21-2	395 435	29-6 16-7 26-0	312 420	38-3 - 6-8 21-7	...	Not examined	+	to	56
14	Heated cabbage juice, un- filtered + 2 g. casein	30 cc.	CJ 8	Average	...	14-3	435	21-3 31-4	431	14-9 30-2	...	...	+	...	64
15	Heated cabbage juice, un- filtered	40 cc.	CJ 11 CJ 12 CJ 13 CJ 14 CJ 18	Average	...	25-3	404 422 389 440 355	31-4 21-6 26-7 17-4 29-7 ...	393 450 400 460 375	30-2 18-3 35-1 20-4 35-6 ill and re- covered	...	+	to	59 121 120 115 62	
		"	CJ 19 CJ 21	335 345	403 370	20-1 7-2	435 395	29-8 14-4	422 ...	25-9 ...	...	+	to	+	94 ...
		50 cc.	CJ 21	Average	...	17-6	...	23-2	398	27-0	+	nearly	...	...	49
			...	...	...	...	...	...	...	...	Average	...	...	...	...

Table I continued

Group no.	Additions to basal diet of oats, bran and water	Amount of ration	No. of animal	Initial weight g.	Wt after 25 days g.	% increase	Wt after 35 days g.	% increase	Wt after 45 days g.	% increase	Histology			Length of life from beginning of expt. to post-mortem, days
											Normal	Incipient	Definite	Acute
16	Normal	...	...	340	450	32.3	500	47.0	535	55.8				
	Dried cabbage juice, green	50 cc.	DC 1	301	360	19.9	390	29.5	395	31.2	...	+	...	85
	"	"	DC 2	313	370	18.2	333	6.3	347	11.1	...	+	...	91
	+ 5 cc. orange juice	"	DC 3	304	350	15.1	360	18.4	365	20.0	+	to	...	82
	Average	...	...	...	...	...	...	...	...	...	...	...	...	...
17	Fresh milk + 10 cc. orange juice	20 cc.	684	334	305	- 8.6	...	...	...	...	...	...	...	...
	"	"	685	335	350	+ 4.4	...	...	...	...	...	...	...	...
	Average	...	...	...	...	...	...	...	...	...	...	...	...	...
18	Raw milk + 10 cc. orange juice	60 cc.	699 M	330	450	36.3	485	46.9	480	45.4	+	...	...	45
	"	"	699 N	333	410	23.1	429	28.8	456	36.9	+ nearly	+	...	45
	"	"	699 O	338	440	30.1	489	44.7	532	57.4	+ nearly	+	...	45
	"	"	699 P	331	424	28.9	470	41.9	490	48.0	+ nearly	...	...	45
	Average	...	...	...	...	...	...	...	...	...	...	...	...	...
19	Aut. milk + 10 cc. orange juice	60 cc.	699 Q	331	455	37.4	516	55.9	532	60.7	+ nearly	...	...	45
	"	"	699 R	330	440	33.3	470	42.4	500	51.5	+ nearly	...	...	45
	"	"	699 S	340	427	25.5	445	30.8	445	30.8	...	+	...	45
	"	"	699 T	340	428	25.8	463	36.1	464	36.4	+ nearly	...	...	45
	Average	...	...	...	...	...	...	...	...	...	...	...	...	...
20	Glaxo = 60 cc. raw milk + 10 cc. orange juice	60 cc.	694	337	441	30.8	466	38.2	474	40.6	...	+	to	45
	"	"	695	334	423	26.6	453	35.9	480	43.7	...	+	...	45
	"	"	696	330	407	23.1	435	31.8	435	31.8	...	+	to	45
	"	"	697	334	403	20.6	417	24.9	450	34.7	...	+	...	45
	Average	...	...	...	...	...	...	...	...	...	...	...	...	...
										37.7				

Histological examination. Only one animal was examined and was found nearly normal.

Steaming cabbage for an hour at 100° does not therefore appear to destroy a perceptible amount of its growth-promoting powers for guinea-pigs.

Green cabbage leaf, heated for two hours at 100°. (Table I, Group 6.)

The cabbage was prepared by being steamed for two hours at 100°.

Group 6. 15 g. daily ration. Five animals received 15 g. daily each and their average percentage increase (37.9 %) for 45 days, is only slightly inferior to the corresponding figure for raw cabbage (40.1 %).

Histological examination. Three of the five animals were examined and showed a normal histology.

Cabbage steamed for two hours at 100° does not appear to be materially damaged as regards its growth-promoting powers for guinea-pigs.

Filtered green raw cabbage juice. (Table I, Groups 7 and 8.)

A number of experiments were planned in order to dissociate the growth-promoting fraction, as far as possible from the proteins and other substances in the actual tissues of the leaf. Juice from raw and heated cabbage, filtered and unfiltered, was used and in one or two cases caseinogen was added to compare the effect.

For the preparation of the raw juice the green portions of the leaf, free of the mid-rib, were minced in a mincing machine, the pulp was enclosed in muslin and pressed out in a hand press. In all cases the animals were allowed to drink the juice if they would; if they would not it was fed by hand. The juice was filtered through paper.

Group 7. 20 cc. daily ration. Two animals received 20 cc. of the raw juice filtered and made some growth, *i.e.* an average of 24.3 % in 45 days; this is a rate of increase well below the normal (55.8 %) and less even than that (29.9 %) made on 5 g. daily of the raw green leaf.

Histological examination. Both animals were examined and one was found to be almost completely normal and the other between normal and "incipient."

Group 8. 30 cc. daily ration. One animal received 30 cc. daily and showed 32.9 % increase in 45 days, an increment which is still nearly 50 % below the normal.

Histological examination. The condition of the rib junctions was distinctly abnormal, being between "incipient" and "definite."

Raw filtered cabbage juice is thus capable of promoting growth but possesses this property in a much less degree than the raw leaf.

Unfiltered green raw cabbage juice. (Table I, Group 9.)

The juice used was the same as in Groups 7 and 8 except that it was unfiltered.

Group 9. 30 cc. daily ration. Only one animal received this ration and its percentage increase for 45 days, *i.e.* 31 %, was almost identical with that

(32.9 %) of the one animal (Group 12) which received 30 cc. of the filtered juice.

Histological examination. The histological result also corresponded with that of the animal in Group 12, being between "incipient" and "definite."

So far as evidence derived from comparison between two single animals is worth anything at all, it points to there being no loss of growth-promoting power in cabbage juice on filtration through paper.

Filtered green heated cabbage juice. (Table I, Groups 10, 11 and 12.)

The juice was prepared by steaming the cabbage at 100° for 20 minutes, folding it in muslin and pressing in a hand press. It was then filtered through a paper filter.

Group 10. 20 cc. daily ration. 20 cc. of the juice was fed to two animals which both showed some rise in weight at the 25th day (average 9.0 %) but this had diminished to 3.6 % on the 35th day. On the 45th day one of the two animals showed a percentage loss over the whole period of 11.2 % while the other showed some loss of what it had previously gained, though there was a net gain over the whole period of 3.9 %. The gain for this animal (No. CJ 6) for 35 days was 11.1 %.

No histological examination was made.

Group 11. 20 cc. juice + 2 g. caseinogen daily ration. The caseinogen was unpurified; it was mixed with a little water and was fed by hand. The quantity 2 g. was selected as being somewhat in excess of the amount present in 60 cc. of cow's milk, a ration of milk well known to be growth-promoting for guinea-pigs.

One animal received the ration but the caseinogen did not affect the result in its case for it just showed maintenance over the whole period of 45 days.

No histological examination was made.

Group 12. 30 cc. daily ration. Only one animal received 30 cc. of the filtered heated juice; for the period of 45 days it showed considerable growth (38.3 %), not equal to the normal (55.8 %), but nearly approaching that for 15 g. raw green cabbage leaf (40.1 %), and rather greater than that for 30 cc. of the raw juice either filtered or unfiltered.

No histological examination was made.

On the 20 cc. dose the juice of heated cabbage appears to be much inferior in growth promoting power to the raw juice, but on the 30 cc. dose this difference is not apparent; individual idiosyncrasy is however so great that very little importance can be attached to the behaviour of single animals, such as are available for comparison of the 30 cc. doses.

Unfiltered green heated cabbage juice. (Table I, Groups 13, 14 and 15.)

The juice was prepared as for Groups 10, 11 and 12 but it was not filtered.

Group 13. 30 cc. daily ration. Two animals received a 30 cc. daily ration

and both of them made about half the normal growth for 35 days, but by the 45th day one animal, CJ 15, had begun to lose slightly and the other, CJ 7, had converted the previous gain into a negative balance of 6.8 %. In such cases as this the dose would appear to be sufficient to start growth but ceases to be so when the animal is larger. It can scarcely be that the animal starts growth on a reserve of its own which gradually becomes exhausted, as seems to be the case with the rat, when suffering from a deficiency of vitamin A, because on some of the diets, such as oats and bran and orange juice (Table II, Group 28), there is absolutely no increase in weight at all but a rapid decline from the start.

Histological examination. The one animal, CJ 15, which made the better growth gave a histological result between "incipient" and "definite"; the other animal was not examined.

Group 14. 30 cc. juice + 2 g. caseinogen, daily ration. This group only contained one animal which made rather better growth than the two animals in the preceding group; at 35 days it showed an increase of 31.4 % but by the 45th day it also had begun to decline and showed a net increase for the whole period of only 30.2 %.

Histological examination. The lesion in the rib junctions was of the third degree, being "definite."

It is unlikely that the rather better growth shown in this case on the addition of caseinogen was due to more than the individual idiosyncrasy of the animal; the addition of caseinogen in Group 2 produced no such effect.

Group 15. 40 cc. daily ration. This group includes seven animals, of which one was ill during the experiment but recovered, the values for it are therefore given but are not included in the averages. The group for each of the three time periods shows a rate of increase about half the normal.

One animal (CJ 21) which showed a low rate of growth had the daily dose increased to 50 cc. after the 35th day but this increase was not reflected in the subsequent rate of growth although the histology was almost perfectly normal.

Histological examination. The histological results were better than those recorded for any of the other cabbage juice groups, excepting Group 7 (20 cc. raw green filtered juice). One animal showed an almost normal histology, three were between normal and "incipient," one was "incipient," one was between "incipient" and "definite" and one was "definite."

Taken together the cabbage juice results indicate that filtration through paper does not affect the growth-promoting properties of cabbage juice but that the juice from the raw leaves is more potent than that from the cooked leaves. As it was observed, in experiments already detailed, that steaming did not appreciably diminish the value of the leaves when fed whole, it must be that the growth-promoting principle does not pass out with the juice on pressure as readily after heating as it did before. Perfectly normal growth was not obtained in any case with the cabbage juice rations but the indica-

tions are that such growth would be secured if larger doses of the juice could be conveniently given to the animals.

Dried cabbage juice. (Table I, Group 16.)

The juice was prepared from steamed cabbage leaves in the manner already described but was not filtered. The yield of juice was about 50 % of the weight of cabbage taken. It was poured into large flat dishes which were placed in a hot room (37°) with an electric fan just beside them. After about 24 hours the liquid had evaporated to a sticky, thick paste and was further dried by the method described by Harden and Robison [1919]. The paste was scraped up and worked up with a knife so as to become full of air bubbles. The aerated mass was placed in a desiccator which was then evacuated. The whole mass swelled and rose up and if left in the desiccator was dry and porous by the next morning and could be broken up into a coarse powder. Frequently however the drying was not complete in one operation and it was necessary to work the mass up and evacuate the desiccator several times. The dry solids generally amounted to about 5 % of the weight of juice taken. The powder was extremely hygroscopic and had to be kept in a desiccator; new batches were made about once a fortnight. For administration to the guinea-pigs, the powder was mixed with a little water and given by hand.

With this ration 5 cc. of orange juice was given daily, as the anti-scorbutic value of the cabbage juice was likely to have suffered seriously in the process of drying.

Group 16. 50 cc. daily ration. The dried cabbage juice was fed to three guinea-pigs in amounts calculated to be equivalent to 50 cc. of the heated juice. Growth was obtained but was less than one-half the normal and was also less than that obtained on smaller doses of the heated juice which had not been dried.

Histological examination. The results were also rather inferior to those on smaller doses of the heated juice. One animal was between normal and "incipient" and two were "incipient."

There is some loss of growth-promoting power on drying cabbage juice but the loss is by no means complete.

Raw cow's milk. (Table I, Groups 17 and 18.)

The milk used in these experiments was very pure, freshly delivered certified country milk; the time of year at which the experiments were performed was unfortunately not the same in every case but the months are given in each case. 10 cc. orange juice daily was given with this and all other milk rations, to supply the anti-scorbutic factor which would otherwise have been insufficient.

Group 17. 20 cc. daily ration, increasing to 40 cc. and later to 60 cc. Dec.-Feb. Two animals received 20 cc. daily and after 25 days one, No. 684, showed a loss of 8.6 % and the other, No. 685, showed a gain of 4.4 %.

After 30 days the amount of milk was increased to 40 cc. daily and after 30 days on this altered ration No. 684 had recovered a very little weight but had not regained its original weight. No. 685 had gained steadily, though not normally, and was allowed to remain on the 40 cc. ration on which it continued to gain slowly for over 80 days. No. 684, however, was altered to 60 cc. daily on which after a little delay it made a sharp gain in weight.

No histological examination was made.

From experiments on the anti-scorbutic values of foodstuffs published elsewhere [Chick and Hume, 1917] it is well known that 60 cc. of autoclaved milk daily gives very good, if not perfectly normal growth in guinea-pigs, when added to a diet of oats and wheaten bran and orange juice. The foregoing experiment was sufficient to orientate and to show that a quantity less than 60 cc. of raw milk will not do so. This amount was therefore chosen as the daily ration in the following experiments, Groups 18, 19, 20 and 21, in which the growth-promoting powers of several kinds of milk were examined.

Group 18. 60 cc. daily ration. June-July. Four animals received 60 cc. daily of the raw milk and made good but not quite normal growth, the average increase for the group for 45 days being 46.9 % as against 55.8 % for the normal.

Histological examination. Of the four animals one was perfectly normal and the remaining three were almost completely so.

It would therefore appear that 60 cc. raw milk a day is almost but not quite a sufficient quantity to promote normal growth.

Cow's milk autoclaved for one hour at 120°. (Table I, Group 19.)

The same milk as that used in Groups 17 and 18 was autoclaved for one hour at 120°.

Group 19. 60 cc. daily ration. June-July. Four animals receiving the above ration showed a rate of growth (44.8 %), almost identical with that (46.9 %) of the animals which received 60 cc. raw milk.

Histological examination. The results were very slightly inferior to those of animals in Group 18, three being nearly normal and one "incipient."

It therefore appears that heating for an hour at 120° in an autoclave does not appreciably damage the growth-promoting power of milk for guinea-pigs.

Dried milk. (Table I, Group 20.)

A well-known brand of dried milk, much used in infant feeding, was employed. It was not more than a month old and was prepared in March and April. The milk was made up so that the liquid should contain 3.5 % of fat but the powder is a proprietary article in which the original composition has been slightly modified. No fine quantitative deductions could therefore be drawn from the result, but it should be possible to say whether drying seriously damages the growth-promoting properties of milk for guinea-pigs or no.

Group 20. 60 cc. daily ration. Four animals received the 60 cc. daily ration and showed over a period of 45 days a rate of growth (37.7 %), slightly inferior to the corresponding rate (46.9 %) for raw milk.

Histological examination. The results were distinctly inferior to those on raw milk, two animals being "incipient" and two between "incipient" and "definite."

Since the dried milk was not prepared from the milk used in the raw milk experiments and since the time of year and consequently the food of the cows were different, it is not possible to attribute the slight inferiority of the dried milk, with certainty, to the fact that it had been dried. It is possible however to say that if drying affects the growth-promoting power of milk for guinea-pigs at all, it only does so very slightly.

Hay autoclaved at 120° for one hour.

No continuous experiments were put up with a daily ration of hay, but six animals receiving oats and bran and varying rations of lemon juice, and ten animals receiving oats and bran and varying rations of germinated peas showed no appreciable growth. Autoclaved hay (about 20 g. daily) was then added to the diets for four days and every animal showed an immediate sharp rise in weight which at once ceased when the hay was taken away again.

After drying and autoclaving for an hour at 120°, therefore, hay still retains considerable growth-promoting power for guinea-pigs, though the above observation gives no indication whatever, whether some of the original growth-promoting power is lost or not.

White cabbage leaf, raw. (Table II, Group 21.)

The first of the non-growth-promoting group of foodstuffs is the raw white heart of cabbage. Very tightly closed cabbage hearts were used and the outer green leaves were stripped off until inside leaves of a uniform pale etiolated greenish yellow were reached.

Group 21. 15 g. daily ration. Guinea-pigs do not willingly eat the heart leaves of cabbage but two were persuaded to do so. One, No. 698, failed altogether to grow and when the experiment ended after 39 days showed a loss of 4.3 %. The other, No. 692, showed a gain of 4.4 % at the 35th day, which was converted into a loss of 2.4 % by the 45th day.

Histological examination. The lesion was well marked being between "incipient" and "definite" for one animal and "definite" for the other.

Since even as little as 5 g. of green raw cabbage have been shown to produce as much as half the normal growth, while 15 g. of white cabbage produce practically no growth at all, it is clear that white cabbage is vastly inferior to green in its growth-promoting properties. When however the state of affairs on a white cabbage ration is compared with that on oats, wheaten bran and orange juice it is apparent that the white cabbage cannot be so completely deficient in growth-promoting properties as is the orange juice. The

Table II

[illegible]

former at least secures something like maintenance for as much as 45 days, whereas on the orange juice there is a heavy loss (average 19.7 %, Group 28, Table II), even in 25 days.

Unfiltered heated white cabbage juice. (Table II, Group 22.)

The juice was prepared in the same way as the juice of steamed green cabbage leaves and the leaves were selected as in the foregoing experiment (Group 21). The juice was not filtered.

Group 22. 40 cc. daily ration. Three animals received the above ration; of these one showed no growth but a negative balance of 4 % even by the 25th day; on the 35th day the negative balance was increased to 12.5 % and before the 45th day the animal was dead. The other two animals, CJ 16 and CJ 17, showed some increase (9 and 10 %) on the 25th day, but by the 35th day CJ 17 was losing weight and was dead before the 45th day; CJ 16 went on gaining a little to the 25th day (14.6 %) but by the 45th day showed a negative balance of 4 % and died shortly afterwards.

Histological examination. One animal was between "incipient" and "definite," one "definite" and one between "definite" and "acute."

Comparison of the 40 cc. dose of the green (Group 15) and of the white cabbage juice shows the great inferiority of the latter. On the green, growth amounting to about half the normal was obtained; on the white, after slight initial growth the animals declined and died in 45 days or less.

Steamed Onion. (Table II, Groups 23 and 24.)

The onion was steamed in a double saucepan for a rather indeterminate time, probably about one hour; it is however known from the experiments with steamed cabbage that steaming even for two hours is unlikely greatly to damage the growth-promoting property if present.

Group 23. 30 g. daily ration. Four animals received this ration and did not give a very uniform result. Two, Nos. 62 and 63, showed a considerable rise, nearly half the normal in the first 25 days but one of these declined and was dead very soon after. No. 63 increased a little further to the 35th day (23.3 %) but then began to decline very slowly, showing a net increase of only 20 % on the 45th day. The other two, Nos. 64 and 65 A, both showed a constantly increasing loss amounting to 10 % in one case and 37.8 % in the other, by the 45th day.

Histological examination. Three of the five animals were examined; one was between "incipient" and "definite" and two were "definite."

Group 24. 60 g. daily ration. One animal received a daily ration of 60 g. on which it showed no gain but almost maintained itself for 45 days.

Histological examination. The lesion was "definite."

Steamed onion does not seem to be wholly devoid of growth-promoting power for guinea-pigs but in most cases even in large rations it produces very little effect.

Swede juice. (Table II, Group 25.)

The juice of the swede turnip (*Brassica campestris*, var. *Napo-Brassica*) was expressed by first grating the swede root on a kitchen grater and then enfolding the pulp in muslin and squeezing out the juice by hand.

Group 25. 20 cc. daily ration. Four animals received the ration and three showed a decline from the start; of these two were dead by the 45th day. The fourth, No. 539, showed an increase of 10.1 % on the 25th day, but by the 35th day the net increase was only 7.6 % and by the 45th day there was a negative balance of 4.3 %.

Histological examination. One animal was "incipient" and three "definite."

Swede juice is evidently almost devoid of growth-promoting power for guinea-pigs in quantities of 20 cc. daily.

Germinated peas. (Table II, Groups 26 and 27.)

Peas were germinated by soaking in water for 24 hours and germinating them in a funnel for 48 hours; the outer coat of the seed was removed and the peas fed to the guinea-pigs whole.

Group 26. 20 g. daily ration. Three animals received the ration for 25 days and just maintained weight; only one received it for the full 45 days and showed a very slight negative balance.

No histological examination was made.

Group 27. 30 g. daily ration. Two animals were used but the experiment only lasted 20 days in one case and 23 in the other, both animals showed slight negative balance.

No histological examination was made.

Germinated peas appear to possess very little growth-promoting power for guinea-pigs.

Orange juice. (Table II, Group 28.)

The oranges were cut across and the juice expressed by hand.

Group 28. 10 cc. ration daily. Four animals received the ration and after 25 days showed a large negative balance, amounting to an average for the group of 19.7 %.

Histological examination. The lesion was very bad, three animals being "definite" and one "acute."

The dose of orange juice given was only a small one, not sufficient to amount to a real test of the growth-promoting properties of orange juice; the result with these four animals is only quoted here to show that there is no gain at all but an immediate and rapid loss on the most deficient type of diet.

DISCUSSION AND CONCLUSIONS.

It is believed that the foregoing experiments indicate that the guinea-pig requires for growth and maintenance a considerable quantity of the fat-soluble accessory factor of McCollum (vitamin *A* of Drummond) in its diet.

Such a conclusion is reached for two reasons, firstly the diets which produced growth were not superior in their protein, salt or caloric value, to those which did not; the difference must therefore lie in their vitamin content, but it is known that the anti-scorbutic and anti-neuritic vitamins were adequately supplied in all cases; the only known dietary constituent which could have been lacking is thus the vitamin *A*.

Secondly, the distribution of the growth-promoting factor revealed in these experiments, and its behaviour under certain forms of treatment, correspond with what is known of the distribution of vitamin *A* and its behaviour in similar circumstances.

To take the first point in more detail, growth occurs on green cabbage juice but not on white nor on swede juice, it occurs on green cabbage leaf (5 g.) but not white (15 g.) nor on germinated peas nor on onions. These sets of rations do not differ materially from one another in salt, protein or caloric value, the difference must rather be that of a body, present only in the minute amounts in which a vitamin is present. If it be granted then that the growth-promoting factor in this set of experiments is probably a vitamin, it is further apparent from the next paragraph that that vitamin corresponds with vitamin *A* in its distribution among foodstuffs and in its behaviour.

The work of McCollum has shown that vitamin *A* is present in green leaves [McCollum, Simmonds and Pitz, 1916] and in the butter fat of milk [McCollum, Simmonds and Pitz, 1916; McCollum and Davis, 1919]; in his book, McCollum [1920, p. 89] makes the generalisation, drawn from various experiments, that it is only present to a small extent in most roots and non-fatty seeds, such as cereals. Osborne and Mendel [1919] show that it is still present in green leaves after drying. Correspondingly it is found in the experiments detailed in this paper, that green cabbage and milk and dried leaves (hay) promote growth in guinea-pigs, while roots, as represented by swede juice and onions (truly an underground stem), and seeds, as represented by peas, fail to do so.

Coutts and Winfield [1918] have shown that milk, after drying, is still a rich source of vitamin *A* for rats and similarly in these experiments, dried milk is found to be little if at all inferior to raw milk in its growth-promoting properties.

Till recently the evidence with regard to the behaviour of vitamin *A* towards heat was conflicting but the position is now greatly cleared up by a contribution of Hopkins [1920], which shows that vitamin *A* is resistant to heat only as long as there is no access of oxygen but that as soon as oxidation is permitted at the time of heating, destruction of vitamin *A* begins to take place. In the present experiments in which milk and cabbage were heated in an autoclave or in a steamer, little or no loss of growth-promoting properties could be detected and correspondingly it is clear that the conditions were not such as would promote oxidation.

Lastly Drummond and Coward have found (Unpublished Experiments) that etiolated and non-green organs of plants are deficient in vitamin *A*,

corresponding with the present results that they are non-growth-promoting for guinea-pigs.

The correspondence is complete as far as the experiments go and there are no discordant facts, but on the other hand the evidence is not quite complete, for in no case can it be said that the *only* difference between a growth-promoting and a non-growth-promoting diet was the presence or absence of vitamin *A*; were it shown for instance that milk on heating with access of oxygen loses at the same time its growth-promoting power for guinea-pigs and its vitamin *A* value for rats, the evidence could be regarded as conclusive.

Prima facie it is extremely probable that the guinea-pig would require vitamin *A* for it is akin to the rat and must be accustomed, in natural conditions, to a diet of greenstuff even richer in vitamin *A* than the diet of the wild rat; such a line of argument is not however really permissible, for the guinea-pig differs, on the other hand, very markedly from the rat in its large need of the anti-scorbutic factor, a need so large that it has probably limited the creature's geographical distribution and made the numerical success achieved by the rat, mouse and rabbit in the struggle for existence, an impossibility for it.

If it can be accepted from these experiments that the guinea-pig is an animal requiring a large supply of vitamin *A*, it provides another animal on which the vitamin *A* value of certain classes of foodstuffs can be tested. The technique of rat feeding for testing the value of substances in vitamin *A* has now been much improved by the work of Drummond and Zilva but it still holds many difficulties and when the vitamin *A* value of a substance is low, so that large quantities of it have to be given, the technique sometimes fails altogether and frequently from the quantitative point of view. It is exceedingly difficult to hand-feed an unwilling rat, it is comparatively easy to do so with a guinea-pig. Further, guinea-pigs do not appear to grow at all if the diet is entirely deficient in vitamin *A*, though perhaps a larger number of experiments is needed, before this can be affirmed absolutely to be the case. If, as is believed, it is the case, then the growth achieved on any diet can be regarded as a measure of the amount of vitamin *A* in that diet and even the first few days of growth or failure can be regarded as the true trend of the whole of the rest of the experiment, without the need of any preliminary depletion period such as it is often customary to use with rats. In this way, vegetables, fruits, extracts of substances and other non-fatty preparations can be tested on guinea-pigs for their vitamin *A* value, but, as far as can be seen, fats cannot be so tested. Fat as emulsion in milk is tolerated by the guinea-pig but for more concentrated fats it appears to have an intolerance; cream and egg yolk were found to be fatal and codliver oil, even in small doses, appeared to have a very deleterious effect; these substances are however ones with which the rat technique is able to deal.

The above experiments were all conducted under the direction of Dr

Harriette Chick by whom I am kindly permitted to collect and tabulate them. Almost every one of those who have ever cooperated with Dr Chick, in her work on deficiency diseases, has contributed to the actual performing of these experiments and I have to thank Dr Dalyell, Miss Rhodes, Miss Skelton and particularly Mrs Barnes and Dr Delf for permission to use them. A number of them have been published already in various papers on guinea-pig scurvy, particularly those of Dr Delf on cabbage [Delf and Skelton, 1918; Delf and Tozer, 1918], the question of the effect of heat on the growth-promoting properties of cabbage is there discussed but it is not thought out of place to quote the same experiments again here.

SUMMARY.

(1) A large number of dietary experiments on guinea-pigs, originally planned to determine the anti-scorbutic values of various foodstuffs, are analysed and the diets tabulated together with the growth of the animals on the diets.

(2) Growth is found to take place when green cabbage (raw or steamed), green cabbage juice, hay and milk (raw, heated or dried) are added to a diet of oats and bran and water. Orange juice was added when the anti-scorbutic value of the ration was insufficient. Growth is found to vary according to the size of the ration given.

(3) Little or no growth is found to take place when raw white cabbage, white cabbage juice, swede juice, orange juice, onion or germinated peas are added to the diet.

(4) It is argued that the diets could only have been deficient in a dietary factor of the rank of a vitamin and that the growth-promoting factor in these experiments corresponds in its distribution among foodstuffs with the known distribution of vitamin A.

(5) As the result of these experiments guinea-pigs are recommended for trial, as suitable for testing the vitamin A value of non-fatty foodstuffs, for which rats are for any reason unsuitable.

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