

XXIII. EFFECT OF HEAT ON THE ANTI-SCORBUTIC ACCESSORY FACTOR OF VEGETABLE AND FRUIT JUICES.

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A KNOWLEDGE of the properties of the accessory food factors is of especial importance in connection with the cooking of food and with its successful preservation. In the case of the best known accessory factors—the water-soluble, the fat-soluble and the anti-scorbutic vitamins—the effect of exposure to heat for shorter or longer periods is not similar, but comparatively little quantitative work has been done on these lines.

The water-soluble factor is now commonly identified with the substance which has been shown to cure polyneuritis of pigeons. Chick and Hume [1917] were unable to detect that the anti-neuritic factor present in wheat germ and in yeast extract was diminished after exposure to a temperature of 100° for two hours, but a temperature of 113° prolonged for 40 minutes destroyed about half the activity of these substances, the rate of destruction being increased at higher temperatures. More recently it has been asserted by McCollum and Simmonds [1918] that the water-soluble vitamin extracted from “navy beans” is destroyed if heated for an hour at 100° in an alkaline medium (0.28 % NaOH).

The fat-soluble vitamin is more sensitive to heat but the evidence on this point is not yet conclusive. According to Steenbock, Boutwell and Kent [1918], butter fat loses nearly all its growth-promoting power after being heated for 4 hours at 100°, and Drummond detected some loss in value after 1 hour's heating at 100° [1919]; moreover the hydrogenation of whale oil at high temperatures completely destroys the fat-soluble content of the original oil [Halliburton, 1919]. On the other hand, the results of numerous observations by workers at the Lister Institute upon milk heated in an autoclave at 15 lb. pressure (*i.e.* at about 120° in absence of air) show that after heating for an hour it still retains the greater part of its growth-promoting efficiency.

More is known as to the effect of heat on the anti-scorbutic accessory factor as it occurs in green cabbage leaves [Delf and Tozer, 1918], and in germinating pulses [Delf and Chick, 1919]. In the former case it was shown that fresh green cabbage leaves lose about four-fifths of their anti-scorbutic value when heated in steam for only 20 minutes to a temperature of 100°.

In the experiments to be recorded in this communication, freshly expressed juices of cabbage, swede and orange were contrasted as to their anti-scorbutic values in the raw state and after heating. In all these cases, it was necessary first to find the minimal daily ration of the fresh raw juice which would just afford protection from scurvy when fed to animals on an otherwise scorbutic diet. The technique was that which has been described at length elsewhere [Chick and Hume, 1917; Delf and Tozer, 1918]. Young guinea-pigs were used as the experimental animals and their basal diet was in all cases oats and bran *ad libitum*, and milk previously autoclaved at a pressure of 15 lb., *i.e.* about 120°. The milk ration was given more liberally than hitherto,—to the extent of 60–90 cc. daily, according to the age and appetite of the animal. This increase of the milk ration was justified by control experiments as yet unpublished. The onset of scurvy is not appreciably affected by the increased allowance and healthier animals more resistant to intercurrent disease can be produced, especially in the case of limiting doses which are often difficult to determine on account of secondary infections, as in the case of the orange juice animals which are quoted in Table V.

In Tables I–IV, the data listed in the columns headed “Histology of rib-junctions” were kindly supplied by Miss F. M. Tozer and the diagnoses made as explained by her in a previous communication [Delf and Tozer, 1918]. In our experience, the histology of the rib-junctions may show a subnormal condition (see No. 933, Table IV) or even definite disorganisation of the tissues (as No. 932, Table IV), when the typical clinical symptoms of scurvy in life have been entirely absent. The histological diagnosis then probably indicates a critical condition (which we have called “incipient”), which, if the experiment were sufficiently prolonged, might develop into the typical disease. Similar pre-scorbutic symptoms have been described by McCarrison in connection with the adrenals and the muscular walls of the alimentary tract, both of which suffer marked changes of structure or function when guinea-pigs are kept for short periods on an entirely scorbutic diet, before the usual symptoms of scurvy can be observed [McCarrison, 1919]. He further regards these pre-scorbutic changes as particularly characteristic of a dietary of low vitamine content, and this is in accord with our impression.

Experiments about to be published by Miss F. M. Tozer prove, as we had formerly expected [Delf and Tozer, 1918], that when the anti-scorbutic element is provided in excess, a diet deficient only in the fat-soluble constituent results in a number of changes in the structure of the rib-junctions of guinea-pigs very similar to those observed in the less severe cases of scurvy. In the experiments described below, the amount of milk consumed by the animals satisfies us that the appearances described are primarily due to the lack of sufficient anti-scorbutic in the diet, even when the animal survived the experimental period in apparent health.

Table I. *Experiments with raw green filtered Cabbage Juice.*

No. of animal	Average amount food consumed daily			Body weight		Symptoms during life	Duration of experiment, days	Post mortem	Histology of rib-junctions	General result
	Ration of juice cc.	Oats & bran g.	Milk cc.	Initial g.	Final g.					
998	10	19	66	325	638	None	86	Normal	"Incipient" scurvy	Protection
999	10	34	77	345	570	"	90	"	"	"
999 C	5	28	76	325	660	"	90	"	Nearly normal	"
999 D	5	32	72	315	580	"	90	"	"	"
999 E	5	47	75	340	617	Tender joints	90	"	"	"
999 F	2.5	45	74	340	605	None	86	"	"	"
999 G	2.5	33	68	335	375*	"	74	Haemorrhage on stomach and caecum	Definite scurvy	Died of visceral haemorrhage. (?) Scurvy
999 H	2.5	48	67	320	620	"	87	Normal	Nearly normal	Protection
1105	1.5	57	89	335	745	"	90	"	"	"
1106	1.5	51	88	335	695	"	90	"	"	"
1118	0.75	30	63	352	405	Knees swollen, tender; very lame	42	Haemorrhages: fractured knees. Enlarged rib-junctions	Not examined	Typical severe scurvy
1119	0.75	28	72	334	445	"	42	"	"	"
1130	1.0	40	79	345	510	One knee swollen: abscess	83	Haemorrhage severe in one knee. Tibia fragile	Incipient—Definite	Mild scurvy
1131	1.0	16	61	335	355	Knees swollen, lame	81	Haemorrhage slight. Bones brittle. Ill—pleural adhesion	"	Scurvy
1132	1.0	24	83	345	675	One knee swollen, teeth loose	81	Teeth loose, otherwise normal	Nearly normal	Protection

* Maximum body weight, 510.

EFFECT OF HEAT ON THE ANTI-SCORBUTIC PROPERTIES OF THE JUICE OF GREEN CABBAGE.

In the case of the cabbage juice, at first only the green outer leaves of fresh cabbages were used. The stouter part of the midrib of each leaf was removed and the remaining part passed through an ordinary mincing machine. The pulp was then wrapped in two thicknesses of stout muslin (mosquito netting) and submitted to a hand-press. The thick green juice was afterwards passed through filter paper, when a clear brownish-green liquid was obtained. Later, by a similar process, the juice was expressed also from the white interior leaves of the cabbages and the freshly prepared green or white juices were fed to different experimental animals.

In the case of the green raw juice, daily rations of 10 cc., 5 cc., 2.5 cc. and 1.5 cc. were all found to give perfect protection from symptoms of scurvy during the experimental period (60–90 days, Table I). It was remarkable that the strongest and best grown animals were on the smallest of these doses (1.5 cc., Fig. 1 *E*), but these two had a large appetite for autoclaved milk (Table I). When this dose was halved and fed to another set of animals, typical and severe scurvy developed after a brief period of normal growth (35–45 days, Fig. 1 *F*). Three animals were subsequently given a ration of 1 cc. fresh juice. Two of these showed slight signs of scurvy at 80 days; the third was normal, except for loose teeth. The minimum raw protective dose is therefore just above 1 cc. daily. Parallel experiments with juice from the white leaves lead to the conclusion that the anti-scorbutic value of the two kinds of juices is not very different, 1.5 cc. affording apparent protection from scurvy.

The juice prepared as described above was placed in small flasks stoppered with cotton-wool plugs and heated in steam at 100° for an hour, or for twenty minutes in the different experiments. A soft whitish coagulum was produced by the heating and this was included in the doses fed to the animals.

Successive experiments with the juice heated for one hour showed that on a daily dose of 5 cc. or less all the animals developed severe scurvy (Table II *a*). Of two animals on a 7.5 cc. dose one remained in perfect health for 90 days when the experiment was terminated, and one developed severe scurvy after 20 days. The latter animal survived in fair condition for 90 days, but was badly crippled in the hind-quarters which at the post mortem showed typical scurvy lesions. Two others on a 10 cc. ration were protected. Evidently a ration of 7.5 cc. is near the limit required for complete protection and perhaps may be regarded as the equivalent of 1 cc. of the raw juice. This indicates a loss of about 80 % of the original value during the hour of heating.

Experiments have also been made in which animals were fed on the juice heated for 20 minutes. Two animals on a 5 cc. dose early developed symptoms of scurvy—No. 1127 on the 26th and No. 1128 on the 28th day. Three other

Table II. *Experiments with green filtered Cabbage Juice heated to 100°.*

No. of animal	Ration of juice cc.	Average amount food consumed daily		Body weight		Symptoms during life	Duration of experiment, days	Post mortem	Histology of rib-junctions	General result
		Oats & bran g.	Milk cc.	Initial g.	Final g.					
1107	3.0	29	64	325	400	Both knees swollen, sore; lame	29	Haemorrhages extensive; enlarged rib-junctions	Scurvy	Severe scurvy
1108	3.0	32	82	330	540	" "	32	" "	Severe scurvy	" "
1100	5	19	69	336	463	" "	90	Haemorrhages extensive; loose teeth	Definite "	Scurvy; some protection
1101	5	40	79	325	525	Left knee swollen, sore, lame.	91	" "	Incipient "	" "
1102	5	31	76	330	527	Both knees swollen, sore, lame	89	Haemorrhages extensive; loose teeth, rib-junctions enlarged	Incipient—definite scurvy	" "
1122	5	19	55	335	366	" "	35	Haemorrhages severe, knees fractured	Severe scurvy	Severe scurvy
1120	7.5	25	83	335	550	No symptoms	90	Normal. 3 rib junctions enlarged	Definite scurvy	Scurvy
1121	7.5	20	79	340	380	Knees swollen: crippled	65	Severe haemorrhages: enlarged knees, enlarged rib-junctions	Definite—severe	Severe scurvy
1139	10	20	83	350	615	Slight soreness, improved	60	Still alive	—	Alive, doing well
1140	10	27	83	320	540	Lame, probable injury	60	Abscess in lung	Not examined	Protected
1127	5	18	75	335	340	Right knee swollen, scurvy position	62	Severe haemorrhages: enlarged knees, enlarged rib-junctions	Not examined	Severe scurvy
1128	5	20	77	328	465	Right knee swollen, lame	89	Severe haemorrhages, bones fragile	Incipient—Definite	Scurvy
1129	7.5	16	83	335	475	Both knees swollen	83	Haemorrhages slight—rib junctions enlarged	Severe scurvy	" "
1137	7.5	15	58	342	385	One knee swollen	54	Haemorrhages, fragile tibia, teeth loose	Incipient	" "
1138	7.5	25	69	340	480	" "	46	" "	—	" "

(b) Heated for 20 minutes.

animals received a ration of 7.5 cc. daily; one of these became scorbutic after 28 days; the other two were killed at 54 and 46 days respectively and both showed distinct signs of scurvy.

These results fall into line with those previously obtained for the green leaves [Delf and Tozer, 1918], in indicating a considerable destruction of the anti-scorbutic value after heating, and the destruction is, if anything, greater in the expressed juice than in the leaf tissue. The experiments with the juice heated for only 20 minutes show how great an amount of destruction of anti-scorbutic substance occurs at the beginning of heating, the loss after an hour being indistinguishable from that after twenty minutes' heating.

THE EFFECT OF HEAT ON THE ANTI-SCORBUTIC PROPERTIES OF THE JUICE OF THE TURNIP-SWEDE (*BRASSICA CAMPESTRIS* var. *NAPO-BRASSICA*).

An account has already been published of results of experiments made by Chick and Rhodes [1918], working in this laboratory, which establish that 2.5 cc. may be regarded as the minimum daily protective ration of raw swede juice. In the winter of 1918-1919, these results were confirmed and extended to the heated juices as summarised in Tables III and IV. I am indebted to Miss D. Gardiner of Girton College for much assistance in the preparation of the juices and in the care of the experimental animals.

The juice was prepared daily by the method (previously adopted by Chick and Rhodes) of rubbing a fresh surface of the root on an ordinary kitchen grater, and squeezing the pulp thus obtained by hand through coarse muslin. Out of four animals given 2.5 cc. daily as the sole anti-scorbutic in their diet, two remained in perfect health throughout the experiment, another was slightly scorbutic but was otherwise in fairly good condition (No. 953, Table III), and the fourth died after 36 days of some intercurrent disease, with accompanying symptoms of mild scurvy. On smaller doses severe scurvy always appeared and on a larger dose protection was achieved in every case.

The fresh swede juice was heated for an hour either in test-tubes plugged with cotton-wool and nearly submerged in a water-bath kept at 80°, or in small flasks also plugged with cotton-wool but heated in a steam chamber at 100°, or in an autoclave at about 10 lb. pressure, *i.e.* about 110°, or at 25 lb. pressure, *i.e.* about 130°. Perfect protection from scurvy was given by a 5 cc. dose of the juice previously heated at either 80° or 100° or even 110°. This dose failed to protect from scurvy if previously heated for an hour at 130° (Table IV), but when the dose was increased to 10 cc., protection was achieved in the case of two experimental animals (Table IV).

Swede juice is thus much more stable at high temperatures than is cabbage juice, double the amount of the raw minimal dose giving protection after heating for an hour at 100° or 110°, whereas with raw cabbage juice about seven times the raw minimal dose is necessary to give this protection after heating for an hour at 100°.

Table III. *Experiments with raw Swede Juice.*

No. of animal	Ration of swede juice cc.	Average amount food consumed daily			Body weight		Symptoms during life	Duration of experiment, days	Post mortem	Histology of rib-junctions	General result
		Oats & bran g.	Milk cc.	Initial g.	Final g.						
938	5	21	69	325	543	None	88	Teeth loose, brittle	Normal	Protection	
939	5	23	53	330	387	"	51	Tibia somewhat brittle	Nearly normal	"	
968	5	21	74	350	487	"	91	Teeth brittle: rib-junctions somewhat enlarged	"	"	
971.	5	24	60	335	512	"	91	Rib-junctions slightly enlarged	"	"	
972	5	52	75	345	617	"	91	Teeth rather brittle	"	"	
952	2.5	18	55	330	325	Joints swollen: infection	36	Muscular and subcutaneous haemorrhages—slight	Incipient scurvy	Infection, mild scurvy	
953	2.5	17	59	330	375	Limbs tender when examined	90	Slight subcutaneous haemorrhages	Scurvy	Protection incomplete, mild scurvy	
970	2.5	19	75	325	530	"	90	Normal	Normal	Protection	
973	2.5	30	75	340	550	None	91	"	Nearly normal	"	
955	1.5	31	70	320	505	"	90	Brittle teeth	"	"	
956	1.5	16	58	320	350	"	45	Haemorrhage on gut	"	"	
962	1.5	12	70	320	388	Scurvy position, knees swollen	90	Subcutaneous haemorrhages; fractured knees, etc.	Scurvy	Scurvy	
978	1.5	15	56	320	340	Limbs very tender on handling	71	Muscular haemorrhages; rib-junctions enlarged	"	"	
979	1.5	21	54	325	395	Lame, body very thin	71	Muscular haemorrhages; rib-junctions enlarged; knees fractured	"	"	
980	0.75	15	58	320	315	Lame; swollen knees	42	Severe haemorrhage, etc.	"	Severe scurvy	
981	0.75	16	59	320	320	"	41	"	"	"	
982	0.75	14	51	325	290	"	34	"	"	"	

EFFECT OF HEAT ON THE ANTI-SCORBUTIC VALUE OF FRESH ORANGE JUICE.

I am indebted to Miss E. M. Hume and Miss A. J. Davey for permission to publish the data quoted in Table V, from which may be seen the results of their experiments with raw orange juice. From the experiments of Miss Hume carried out in 1917, it will be seen that 3 cc. of the juice affords ample protection from scurvy in young guinea-pigs and that fairly good growth was made when autoclaved milk was included in the dietary. The results of a dose of 1.5 cc. were variable, only five out of eleven animals receiving adequate protection from scurvy (Table V, Nos. 720, 721, 723, 744, and 1000). Of these only No. 1000, which had the largest appetite for milk, made a practically normal growth curve (Fig. 4 B). Two of the animals (Nos. 743 and 1001) were presumably in a "pre-scorbutic" condition, showing histological deformities without any clinical symptoms of scurvy. On the whole, we may conclude from these experiments that 1.5 cc. is the minimal protective ration of the freshly squeezed juice from oranges in good condition when fed to animals which can be kept free from other forms of disease.

Freshly squeezed orange juice was heated for an hour at three different temperatures. At 70° in a water-bath as for the swede juice; at higher temperatures in a steam chamber or autoclave as already described for the other juices.

One case of protection was observed on a 1.5 cc. dose, heated at 70°, but this was probably an exceptional animal since a larger ration given later in the year failed to give complete protection (3 cc., Table VI). Animals were kept successfully in good health throughout the experimental period both on 3 cc. rations and on 1.5 cc. rations heated at 100°; but in the former case two out of four animals were found to exhibit post mortem and histological signs of scurvy (perhaps attributable to the difficulty of getting good oranges at that time), whilst in the latter case two out of three animals showed similar signs of a scorbutic or rather pre-scorbutic condition (weight charts, Fig. 4 C, D). Two animals on a 3 cc. dose heated at 130° were protected from scurvy and a third nearly protected, but three other animals on a 2 cc. ration of this juice developed severe scurvy. Orange juice is therefore still more stable than swede juice, less than double the raw minimal dose affording protection after an hour of heating at 130°.

This stability of the orange juice to heat suggested the possibility that the acidity present in the juice might have a stabilising effect on the anti-scorbutic substance, and led to experiments carried out mainly by Miss B. F. Runge in which the juice was nearly neutralised with 10 % sodium carbonate prior to heating. The alkaline solution was added drop by drop until the liquid was just acid to a standard solution of dibromo-*o*-cresolsulphonephthalein (P_H 5.2-6.8). The residual acidity of the juice was then about the same as the slight natural acidity of the swede and cabbage juices used in the other experiments.

Table IV. *Experiments with Swede Juice heated for 1 hour.*

No. of animal	Ration of swede juice cc.	Temp. of juice heating °C.	Average amount food consumed daily			Symptoms during life	Duration of experiment, days	Histology of rib-junctions	General result		
			Oats & bran g.	Milk cc.	Body weight Initial Final g.						
931	5	80	22	67	337	410	No symptoms	84	Haemorrhage on small intestine	Incipient scurvy	Protection, possible infection
932	5	80	21	71	355	465	"	82	Normal	Scurvy	"
940	5	80	13	66	322	390	Lung trouble; scurvy also	92	Subcutaneous and muscular haemorrhages; bones fragile	"	Partial protection
933	5	100	18	69	320	480	No symptoms	90	Teeth brittle	Incipient scurvy	Protection
934	5	100	20	72	340	517	"	92	"	"	"
941	4	100	26	68	330	412	"	90	One rib-junction much enlarged	"	"
943	5	110	10	60	330	370	Limbs tender after 38th day	85	Lungs inflamed; intestinal ulcers	Nearly normal	"
944	5	110	12	75	335	485	"	91	Teeth brittle	Incipient scurvy	"
945	5	110	16	68	322	485	"	92	Rib-junctions ridged	"	"
936	10	130	18	67	350	498	"	88	Slight muscular haemorrhages; intestinal ulcers	"	"
937	10	130	30	74	310	540	"	90	Teeth brittle	Normal	"
974	5	130	13	53	341	320	Scurvy position, lame after 21st day	33	Muscular haemorrhages, fragile bones, etc.	Not examined	Typical acute scurvy
975	5	130	15	58	340	335	"	34	"	"	"
976	5	130	17	58	320	287	"	31	"	"	"

Three animals given 3 cc. of this juice heated for an hour at 100° and three others given only 1.5 cc. daily were successfully protected from scurvy during the experimental period, the condition of those on the larger ration being rather better than of those on the smaller. Neutralising nearly all the excess of acid in orange juice therefore does not appreciably reduce the stability of the anti-scorbutic constituent of the juice at a temperature of 100°.

Experiments were also made with the juice of oranges which had been canned and stored at laboratory temperatures for five months. The canning process involved heating in closed cans for 20–30 minutes, the temperature gradually rising from about 80° to 100° and remaining at 100° for not more than five minutes. A certain amount of water was added to the fruit after packing the cans and this was included in the juice afterwards expressed by hand from the fruit when the cans were opened. Allowing for the added water, a dose equivalent as nearly as possible to 1.5 cc. of the fresh juice was given daily to three experimental animals and afforded them adequate protection from scurvy for 88 days, when the experiment was terminated. Canned oranges produce a bitter juice and are probably of no commercial value, but this result with fruit in which the value of the raw juice is well known indicates the possibilities of investigations with other fruit juices.

DISCUSSION OF RESULTS.

Experiments have been described in which the juice of fresh cabbage, swede and orange has been fed to animals as the sole anti-scorbutic element in a basal diet of oats and bran *ad libitum*, and 60–90 cc. autoclaved milk daily. From these experiments it can be affirmed that under these conditions the minimal daily dose of the raw juice for the adequate protection from scurvy of young guinea-pigs is about 1.0 cc. cabbage, 2.5 cc. swede, and 1.5 cc. orange juice respectively.

When these juices are subjected to temperatures up to 130° a corresponding reduction of their anti-scorbutic properties appears. This reduction is greatest in the case of cabbage juice and is least in the case of orange juice. After being heated at 100° for an hour, at least 7½ times the raw dose in the case of cabbage, or twice the raw dose in the case of swede, is necessary to give protection from scurvy, whereas orange juice does not appear to have deteriorated appreciably during this period of heating. Only at 130° was any definite loss of value detected in the orange juice; 2 cc. was then an insufficient dose for protection, but 3 cc. gave the necessary protection after heating at this temperature.

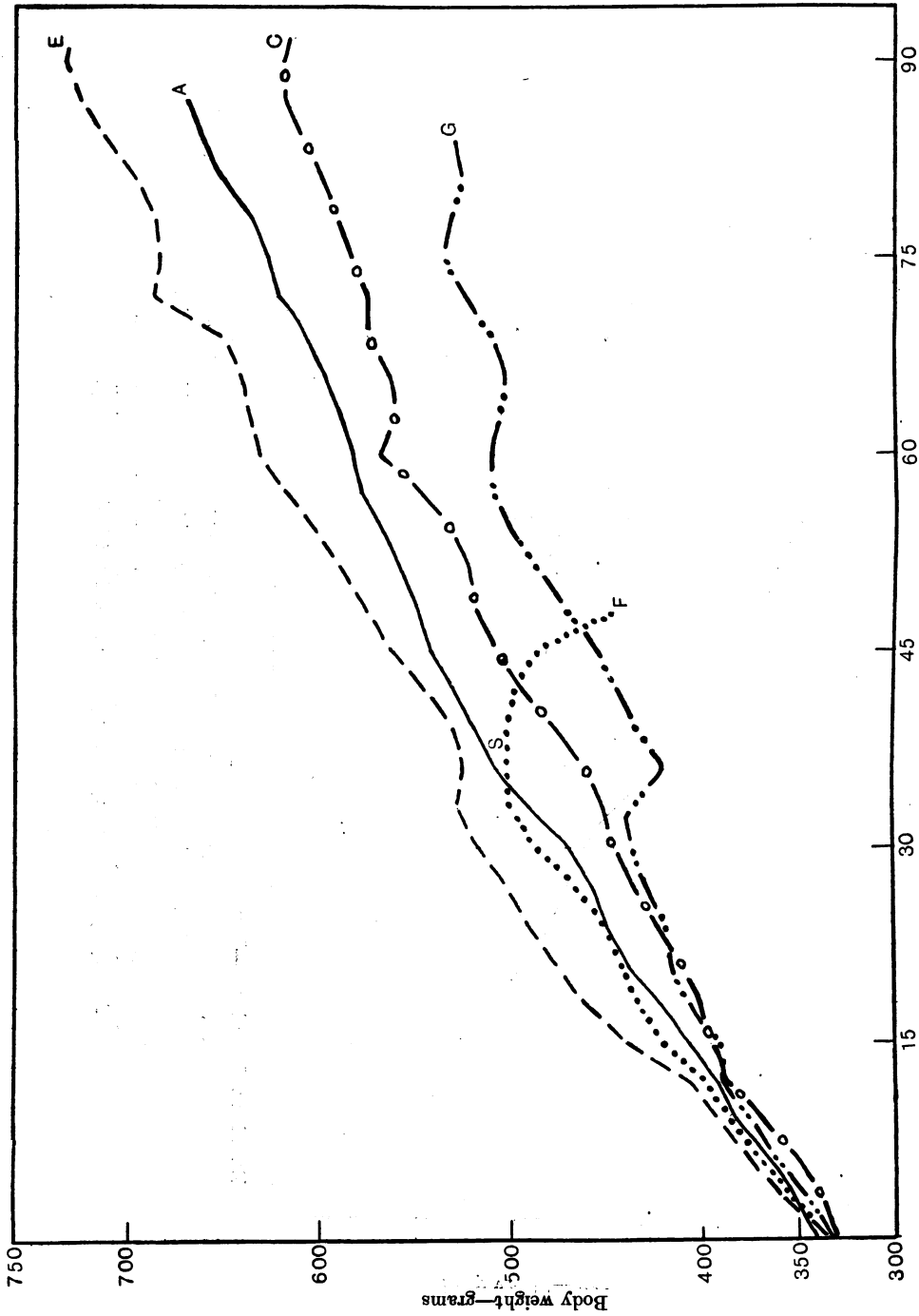
In considering these results, it is interesting to find that orange juice, which is the most stable of the juices tested, is also the most acid in reaction. As long ago as 1912, Holst noticed that fruit juices were more heat-stable than vegetable juices, and he suggested that the stability was due to the presence of acid [Holst and Frölich, 1912]. That the hydrogen ion concentration may have an important influence on the stability of this vitamine is

Table V. *Experiments with raw Orange Juice: basal diet oats, bran and autoclaved milk.*

No. of animal	Date of starting experiment	Ration orange juice cc.	Averages amount food consumed daily		Symptoms during life	Duration of experiment, days	Post mortem	Histology of rib-junctions	General result
			Oats & bran	Milk					
79	21.i.17	5	35	60	290	420	Normal	Incipient scurvy	Protection
111	25.i.17	5	30	74	315	520	Rib-junctions slightly ridged	Normal	"
239	1.v.17	3	52	55	365	598	None	"	"
240	3.v.17	3	65	64	300	365	Practically normal	Nearly normal	"
1013	13.i.19	3	52	76	336	605	Knees swollen, tender after 53rd day	Normal	"
1014	25.i.19	3	41	60	342	514	None	"	"
720	9.iii.18	1.5	63	57	342(490)	378	Intestine probably infected; spots on liver	Nearly normal	"
721	11.iii.18	1.5	85	60	335	510	Normal	Incipient scurvy	"
722	13.iii.18	1.5	66	54	360	372	Haemorrhages in stomach and caecum; two rib-junctions enlarged	Incipient scurvy	Scurvy
723	13.iii.18	1.5	39	51	336(473)	245	Tibia firm, femur brittle; digestive tract unhealthy	Definite scurvy	Protection
742	11.v.18	1.5	25	53	340	260	Fragile bones; intramuscular haemorrhages on knees	Severe scurvy	Scurvy
743	17.v.18	1.5	43	59	341	318	Bones slightly fragile	Definite scurvy	Protection—died of unknown illness
744	6.vi.18	1.5	49	58	337(472)	368	Teeth brittle	Incipient scurvy	Protection
1000	6.i.19	1.5	56	78	337	651	Normal	Nearly normal	Partial protection
1001	9.i.19	1.5	43	58	334	287	Tibiae fragile; rib-junctions rigid, one heavily	Severe scurvy	Partial protection
1002	11.i.19	1.5	36	62	339	340	Liquid in pleural cavity; haemorrhages on caecum	Definite scurvy	Partial protection; pneumonia
1003	11.i.19	1.5	24	53	338	263	Dark inflammatory patches on lungs	"	Partial protection; lung trouble
745	14.vi.18	0.5	37	58	340(474)	400	Haemorrhages in muscles of knees; rib-junctions slightly enlarged	Incipient scurvy	Slight scurvy
1025	3.iii.19	0.5	22	64	339	267	Swollen knees on 22nd day, scurvy position	Severe scurvy	Severe scurvy
1026	4.iii.19	0.5	22	63	331	360	Swollen knees after 38 days, scurvy position	Definite scurvy	Scurvy
1027	6.iii.19	0.5	30	56	331	309	Knees swollen after 34 days	"	"
1028	8.iii.19	0.5	24	59	330	464	Knees swollen after 43 days	"	"
1029	11.iii.19	0.5	40	59	340	311	Knees swollen after 29 days	"	"
1030	17.iii.19	0.5	18	59	343	230	Knees swollen after 20 days	Severe scurvy	Severe scurvy

Table VI. *Experiments with heated Orange Juice: basal diet oats, bran and autoclaved milk.*

No. of animal	Date of starting experiment	Ration of orange juice cc.	Temp. of heating °C.	Average amount food consumed daily				Symptoms during life	Duration experiment, days	Histology of rib-junctions	General result
				Oats & bran g.	Milk cc.	Initial g.	Final g.				
871	10. vi. 18	3	70	37	59	330	550	Slight muscular haemorrhage; fragile bones	90	Severe scurvy	Scurvy, but good health
887	23. iv. 18	1.5	70	37	57	331	652	Slight haemorrhage on intestine	94	Nearly normal	Protection
888	23. iv. 18	1.5	70	29	47	326	318	" "	26	" "	Protection; death from unknown cause
905	7. ix. 18	3	100	36	60	350	593	Teeth loose; tibiae brittle	90	Incipient scurvy	Protection
906	7. ix. 18	3	100	18	60	345	500	Rib-junctions swollen; slight muscular haemorrhage	90	Definite scurvy	Partial protection; slight scurvy
949	1. xii. 18	3	100	22	68	320	630	No symptoms	90	Normal	Protection
950	2. i. 19	3	100	24	70	325	598	" "	89	" "	" "
986	25. iv. 19	1.5	100	22	75	320	610	" "	92	One rib-junction enlarged; the rest normal	Protection; trace scurvy (?)
987	21. iv. 19	1.5	100	14	63	328	485	" "	94	Slight muscular haemorrhage	" "
988	25. iv. 19	1.5	100	22	74	320	670	" "	89	Rib-junctions slightly enlarged	Nearly normal
951	4. i. 19	3	130	10	60	325	335	Knees tender, a little lame	72	Emaciated; lungs inflamed	Definite scurvy
959	13. i. 19	3	130	17	63	320	525	Knees tender	90	Rib-junctions slightly ridged	Normal
960	19. i. 19	3	130	15	66	340	402	" "	90	" "	Incipient scurvy
983	8. iv. 19	2	130	23	60	325	350	Knees much swollen; very painful	40	Severe haemorrhages; fragile bones	Severe scurvy
984	8. iv. 19	2	130	16	69	330	430	" "	78	" "	" "
985 A	9. iv. 19	2	130	15	72	320	450	" "	85	" "	" "
B. Heated nearly neutralised orange juice.											
989	17. v. 19	3	100	17	74	325	535	No symptoms	92	No symptoms	Incipient scurvy
990	17. v. 19	3	100	23	74	325	670	" "	92	Fatty and subcutaneous tissue injected	Normal
991	21. v. 19	3	100	27	76	322	600	" "	90	Normal	Incipient scurvy
992	27. v. 19	1.5	100	21	72	323	515	" "	89	Jaw and lower teeth somewhat brittle	" "
993	27. v. 19	1.5	100	20	72	325	585	" "	90	Rib-junctions all slightly enlarged	" "
994	27. v. 19	1.5	100	20	74	330	510	" "	89	Normal	" "



Duration of Experiment—Days

Fig. 1. Weight charts showing growth of animals fed on oats, bran, autoclaved milk and raw or heated green cabbage juice. A = Mean weight chart of four animals: showing standard growth on normal diet of oats, bran and 30 g. fresh cabbage daily and water. C = " " Nos. 989 C, D, E, on 5 cc. raw filtered green cabbage juice. E = " " Nos. 1105, 1106 on 1.5 cc. " F = Weight chart of No. 1119 on 0.75 cc. raw filtered green cabbage juice. " At S symptoms of scurvy first appeared. G = " " No. 1120 on 7.5 cc. green filtered cabbage juice heated for an hour at 100° C.

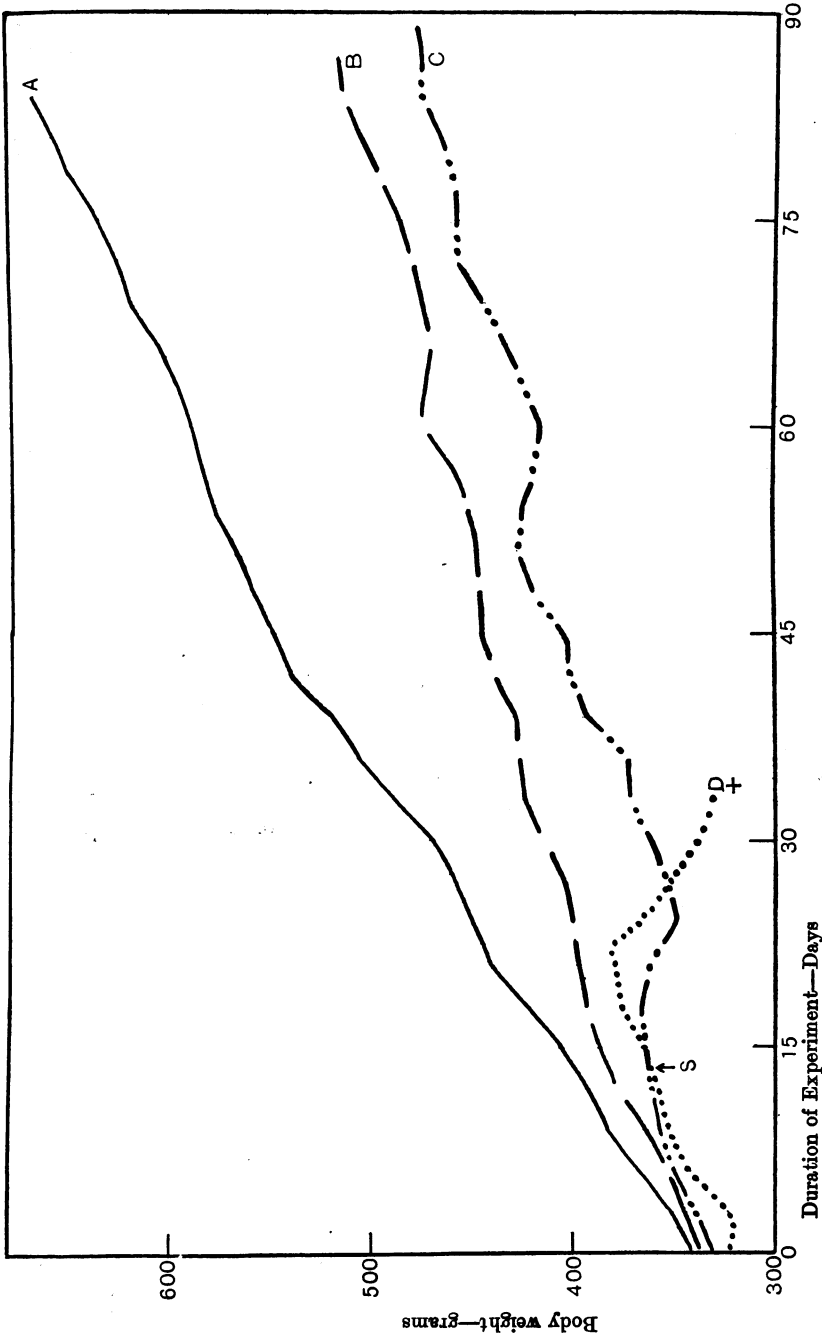


Fig. 2. Weight charts of animals on oats, bran, autoclaved milk and raw swede juice.
 A = Standard weight chart on diet of oats, bran, water and 30 g. fresh cabbage daily.
 B = Mean weight chart of Nos. 938, 968, 971, 972, on a ration of 5 cc. raw swede juice.
 C = " " Nos. 953, 970, 973, " " 2.5 cc. " "
 D = " " Nos. 980, 981, 982, " " 0.75 cc. " "
 At S symptoms of scurvy first appeared; at + No. 982 died of scurvy.

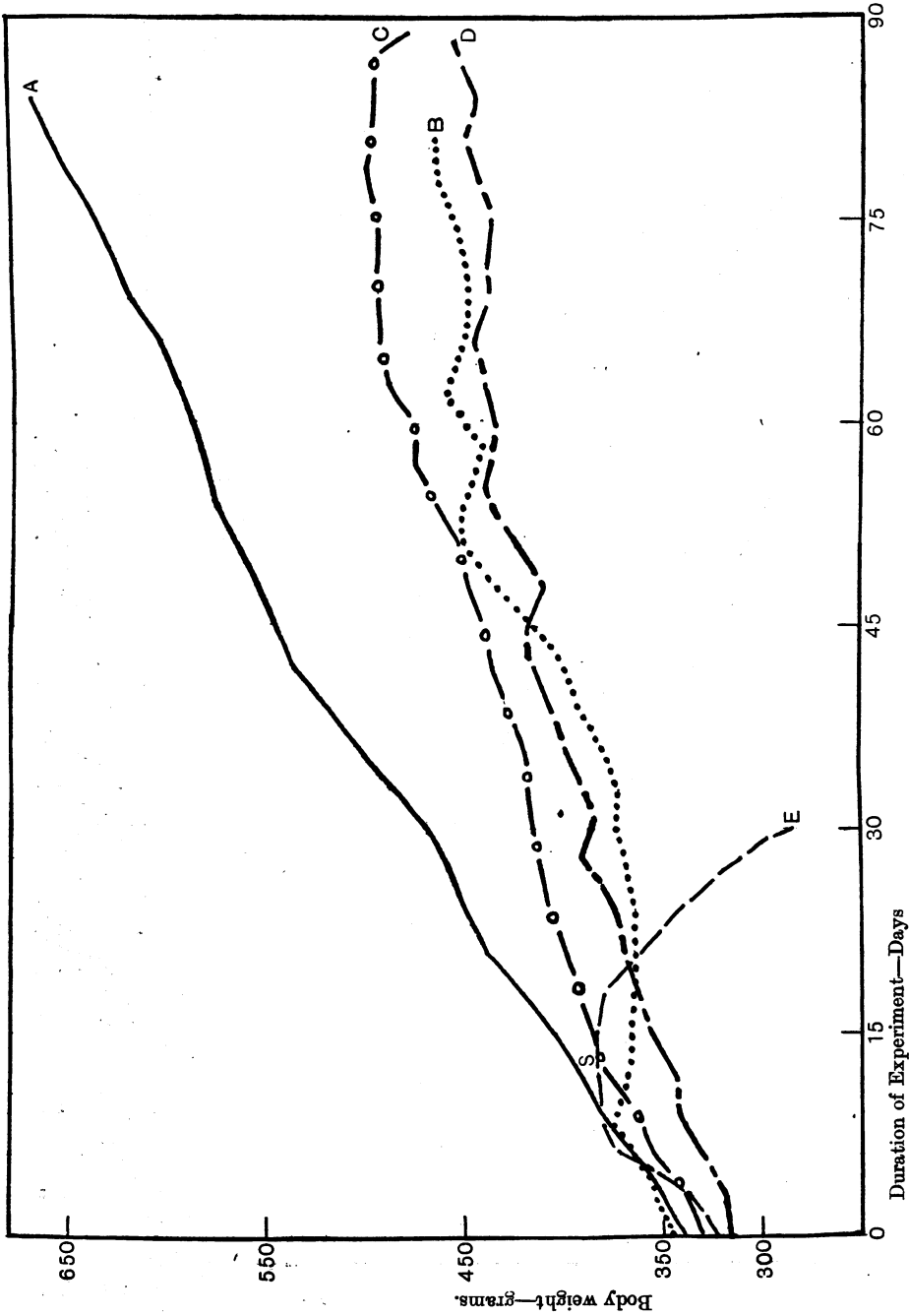


Fig. 3. Weight charts of animals fed on oats, bran, autoclaved milk and heated swede juice.

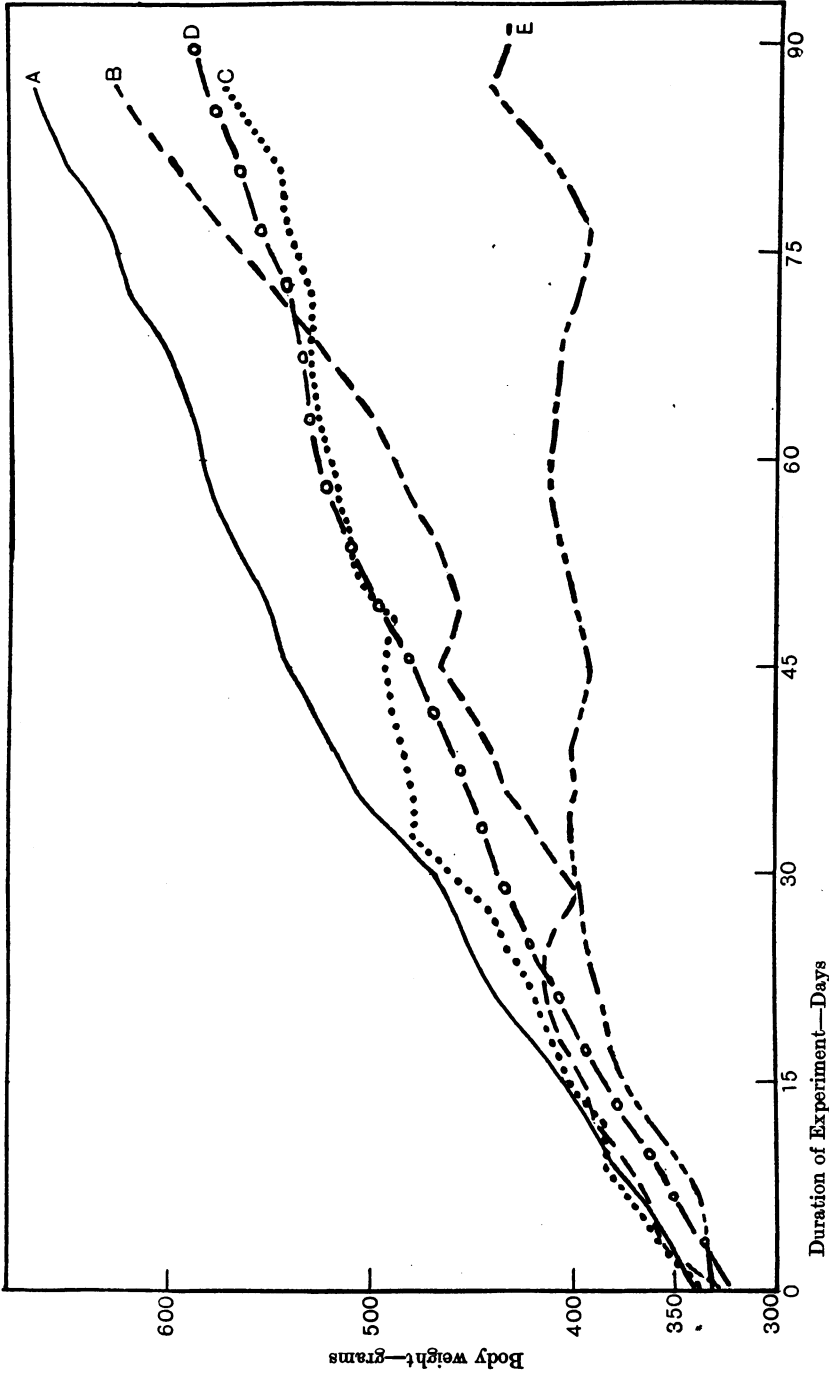
A = Standard weight chart of normal growth (as for Figs. 1, 2).

B = Weight chart of No. 932 on 5 cc. swede juice heated for 1 hour at 80° C.

C = Mean weight chart of Nos. 933, 934, 941 A, on 5 cc. swede juice heated for 1 hour at 100° C.

D = " " Nos. 937, 961, on 10 cc. swede juice heated for 1 hour at 130° C.

E = Weight chart of No. 977, on 5 cc. swede juice heated for 1 hour at 130° C. At S symptoms of scurvy first appeared.



Duration of Experiment—Days

Fig. 4. Weight charts showing growth of animals on a diet of oats, bran, autoclaved milk and a ration of raw or heated orange juice daily.
 A = Standard curve of normal growth (as in Figs. 1-3).
 B = Weight chart of No. 1000, on 1.5 cc. raw orange juice (Table V).
 C = Mean weight chart of Nos. 949, 950, 905, 906, on 3 cc. fresh orange juice heated to 100° C. for an hour.
 D = " " " " Nos. 986, 987, 988, on 1.5 cc. fresh orange juice heated to 100° C. for 1 hour.
 E = " " " " Nos. 959, 960, on 3 cc. fresh orange juice heated to 130° C. for 1 hour.

shown by the experiments of Harden and Zilva [1918], who found that orange juice which was made only slightly alkaline lost almost immediately its anti-scorbutic value. In the experiments described above, the raw juices of cabbage and swede have about the same hydrogen ion concentration, but this slight acidity is accompanied by very different degrees of stability in the two cases. The greater stability of the orange juice to heat was maintained even when the juice was nearly neutralised before heating.

A further point of difference between the three juices tested may be found in their effect on the growth of the experimental animals. A glance at Figs. 1-4 (pp. 223-6) will give the general impression that the cabbage juice animals were on the whole the most satisfactory in this respect, and the variability in the consumption of milk does not altogether account for this¹. It is well known that green vegetables have growth-promoting as well as anti-scorbutic properties and other workers in this Institute have obtained evidence (as yet unpublished) that this growth-promoting power is also shared to some extent by the expressed juice. No appreciable growth is obtained, however, when guinea-pigs are fed on either cabbage, swede or orange juices in doses up to 10 cc. if there is no other source of fat-soluble substance in the diet. In the above experiments therefore, the apparent limitation of growth in the case of certain groups of animals in spite of their considerable allowance of milk suggests that a low ration of anti-scorbutic in a diet may also limit the availability of the fat-soluble substance to the animal, even when protection from scurvy has been secured. In the case of the animals to which swede juice was fed (Table III), if we except No. 939 (on 5 cc.) and No. 952 (on 2.5 cc.), both of which died early in the course of the experiment with some unknown complaint, the amount of milk consumed by the two lots is about the same, but the average rates of growth of the animals are different (Fig. 2 *B*, *C*); whilst in both these cases the growth of none of the animals approached that of the normal growth on a cabbage diet (Fig. 2 *A*). In the case of animals on 3 cc. heated orange juice (Table VI), the consumption of milk was nearly equal in the cases of the two groups of animals, but those on the juice heated to 130° made very little growth although the ration of juice given proved to be just above the scurvy limit (Fig. 4 *C*, *E*). Growth seems therefore to be affected by the limitation of the anti-scorbutic element in the diet apart from the appearance of definite symptoms of scurvy and apart from deficiency in the growth-promoting vitamins.

With regard to the somewhat unexpected stability of swede and orange juice at temperatures above 100°, this is parallel with what was previously found with green cabbage leaves. It must be remembered that the heating at these temperatures was done in a closed autoclave in the absence of air. This may well affect the rate of destruction either directly by retarding oxidation or indirectly by the production of stabilising bodies. The rather

¹ Cp. Nos. 970, 973, Table III with 999 *F*, *H*, Table I, both on 2.5 cc. doses; or Nos. 938, 968, 972, Table III with 999 *C*, *D*, *E*, Table I, both on 5 cc. doses.

surprising stability at 130° in the absence of air suggests that there may be advantage in adopting methods of canning fruit or vegetables at temperatures above boiling-point for as short a time as possible to ensure sterility. Further investigations into the value of canned products would appear to be desirable.

In conclusion I have to thank Dr Harriette Chick for suggestions and advice especially during the earlier stages of the investigation, and Miss F. M. Tozer for permission to quote the results of her histological investigations of the rib-junctions of the animals used in these experiments.

REFERENCES.

- Chick and Hume (1917). *J. Soc. Trop. Med. Hyg.* **10**, 141.
Chick and Rhodes (1918). *Lancet*, ii, 774.
Delf and Chick (1919). *Biochem. J.* **13**, 201.
Delf and Tozer (1918). *Biochem. J.* **12**, 416.
Drummond, J. (1919). *Biochem. J.* **13**, 81.
Halliburton and Others (1919). *J. Physiol.* **52**, 328.
Harden and Zilva (1918). *Lancet*, ii, 320.
Holst and Frölich (1912). *J. Hyg.* **72**, 1.
McCarrison (1919). *Indian J. Med. Research*, **7**, 188.
McCollum and Simmonds (1918). *J. Biol. Chem.* **33**, 55.
Steenbock, Boutwell and Kent (1918). *J. Biol. Chem.* **25**, 517