XXII. THE ANTI-SCORBUTIC VALUE OF DRY AND GERMINATED SEEDS.

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SCURVY is always a menace to any community of people who are separated from adequate supplies of fresh food, whether in tropical or arctic climates. Such it proved to be to our army in Mesopotamia in 1915 and 1916 (*Mesopotamia Commission Report*, 1917), especially among the Indian troops. In consequence of this outbreak, a group of investigators in this Institute undertook an investigation of foodstuffs to determine their relative value as anti-scorbutics and especially to discover some effective material which could easily be transported and would not readily deteriorate. It has been known for centuries by practical experience of human diets, and has recently been proved experimentally [Holst and Frölich, 1912; Delf, 1918] that vegetables of high anti-scorbutic potency become useless for the prevention of scurvy if they are dried and thus rendered suitable for transport.

The most likely solution of the problem had already been indicated by Fürst [1912]. In the series of classical enquiries into experimental scurvy initiated by Holst in the University of Christiania, the work of Fürst showed that anti-scorbutic stuff was developed in substantial quantity during the first days of germination of various seeds. Peas, beans and other pulses, containing as they do only about 10 % of water, are eminently suited for transport, do not readily deteriorate and can be germinated for use where and when required. They form moreover a large part of the normal diet of the Indian soldier.

Experiments at this Institute with guinea-pigs on a diet consisting mainly of peas or lentils confirmed Fürst's results, and these preliminary trials were published in brief by Chick and Hume [1917] in whose paper directions were given for the germination of the dry seeds on active service. The subject appeared, however, to deserve more detailed examination, especially in respect of the anti-scorbutic value of germinated seeds in comparison with other foodstuffs and of its destruction by ordinary methods of cooking. The work undertaken to complete and amplify the earlier trials is set out in the following pages. Protocols of the individual animal experiments are given in the tables, which in many cases contain results of the earlier and later experiments side by side.

I. EXPERIMENTS OF FÜRST.

Guinea-pigs fed exclusively on cereals and water, the routine "scurvy diet" in Holst's laboratory, die of acute scurvy in 20 to 30 days. Fürst [1912] showed that when dry pulses were substituted for the cereals, scurvy also occurred, though in a less acute form, and life was to some extent prolonged. Thus five animals on a diet of dry yellow peas and water died of scurvy in 29 to 55 days; on dry green peas three animals out of four showed definite scurvy in 57 to 64 days, while the fourth lived 131 days without signs of the disease. No growth occurred in any of these animals, which indeed lost weight considerably. Fürst's experiments with germinated seeds were made with barley, oats, peas and lentils. These were soaked in water for 24 hours and afterwards kept moist with access of air and allowed to germinate at summer temperature for two or three days, when sprouts were distinctly visible. In this condition the seeds showed distinct anti-scorbutic properties. Thus two animals fed on water and sprouted barley lived 52 and 92 days without any sign of scurvy and four animals on sprouted oats lived 35 to 78 days and only one had symptoms. With peas and lentils even better results were obtained: four animals on germinated green peas lived 143 to 182 days and four on germinated lentils 89 to 183 days without signs of scurvy.

Growth of animals was also defective with germinated seeds, increase of weight taking place only with green peas.

Fürst also made the interesting observation that if germinated barley was dried at 37° the anti-scorbutic properties gained during germination were lost.

II. METHODS ADOPTED IN THE PRESENT RESEARCH.

The method used in this department in the study of experimental scurvy differs from that employed by Holst and his colleagues in two important points. In the first place the uneaten residues of food are collected and weighed each day; so that the actual intake of food is measured as well as the amount of the offered ration; this seems to us essential in any quantitative nutritional experiments. In the second place a daily ration of cow's milk, autoclaved for one hour at 120° C. (to reduce its original content of anti-scorbutic vitamine to a minimum) is in many cases added to the diet to improve its nutritive value without appreciably affecting the supply of antiscorbutic accessory factor.

In the earlier experiments, the basal diet of the guinea-pigs was water with oats and bran *ad libitum*, on which diet death from acute scurvy was found to take place in from 20 to 30 days. To this diet a weighed ration was added daily of the substance whose anti-scorbutic value was to be investigated. When peas or lentils, dry, soaked or germinated, were added to this basal diet, there was little or no growth, irrespective of whether scurvy was or was not prevented. The addition of autoclaved milk, however, helped to supply the missing growth-promoting substances. On a diet of oats, wheaten bran and autoclaved milk, normal growth and development took place if an adequate

amount of the anti-scorbutic factor were supplied in addition. When the supply of the antiscorbutic factor was inadequate, the initial growth was soon checked, scurvy symptoms were observed and death from scurvy occurred in a time only slightly longer than when the diet consisted of grain and water only. (See Table VI.)

The symptoms of guinea-pig scurvy have recently been described in detail [Chick, Hume and Skelton, 1918; Delf, 1918] and it is unnecessary to recapitulate them here.

Dry "Clipper" brand green peas (*Pisum sativum*) and ordinary brown lentils (*Lens esculenta*), unsplit and unhusked, were selected for the experiments. The latter, as "masoor dhal," is a common article of the native Indian diet. Most of the work was done with whole lentils purchased in the ordinary market; they were obtained with difficulty as it is usual to mill, split or crush them before distribution, which practice almost completely destroys their capacity for germination [see Greig, 1917]. Husking of lentils greatly improves their appearance, as the removal of the brown skin leaves a product orange pink in colour and of appetising appearance.

The guinea-pigs used were young growing animals of about 320 to 350 g. weight. They were mostly bred in the Department, to obtain a regular standard of health and vigour.

III. EXPERIMENTS SHOWING THAT DRY PEAS HAVE SMALL ANTI-SCORBUTIC VALUE.

In our experience, young guinea-pigs cannot be induced to consume any considerable amount of either peas or lentils in the dried state. An attempt was made to overcome this difficulty by offering it in the form of meal. The dry peas were ground in a coffee mill and the resulting meal sifted free from the finer particles (which are apt to cake together in a sticky mass when wetted) and shaken free of the skins. The coarse meal thus obtained was moistened with water and allowed to stand for half an hour before being offered to the animals. It was not eaten rapidly, so that some, at least, of the meal was in the soaked condition for some hours before being actually consumed. Residues left over from a day's ration were washed free from particles of the peat on which the animals were bedded, air-dried and weighed.

Three animals were placed upon a diet of oats and bran *ad lib.*, autoclaved milk to 60 cc. and 15 g. dried ground peas (Table I). In no case was this ration of pea-meal eaten regularly and the average amount consumed daily varied from 7 to 10 g. One animal (349) died after 31 days with every symptom of acute scurvy. The other two (347, 348) developed scurvy which became chronic. No. 348 (*B*, fig. 1) consumed a daily average of 9.6 g. pea-meal; after 44 days it became lame, developed loose teeth and had to be fed daily by hand with milk and little pellets of the moistened pea-meal. It continued for a long time in this condition and when killed by chloroform at the end of 105 days was found to be fairly well nourished. There were, however,

Table I. *Experiment with dry peas.*

Number of animal	Ration offered	Average amount consumed daily			Symptoms during life	Duration of experiment, days	Post-mortem	Histology of rib-junctions	General result
		Peas g.	Oats and bran g.	Milk cc.					
348	15 g. dry pea-meal	9.6	17	52	Initial 325 Final 410 Soreness, becoming very lame, joints swollen Soreness, swollen limbs	105	Bones brittle, rib junctions enlarged, extensive old and recent haemorrhages Bones fragile, rib junctions enlarged, muscular and visceral haemorrhages	Scurvy	Chronic scurvy
349	"	8.8	20	37	340 315 Soreness of limbs, scurvy position	31	Bones brittle, rib junctions not ridged, haemorrhages not severe	Severe scurvy	Died of severe scurvy
347	"	8*	20	40	350 345 Soreness of limbs, scurvy position	30 } 59 } 104 } 15 }	Bones brittle, rib junctions not ridged, haemorrhages not severe	? Scurvy†	Scurvy

Table II. *Experiments with soaked peas.*

Number of animal	Ration offered	Average amount consumed daily			Symptoms during life	Duration of experiment, days	Post-mortem	Histology of rib-junctions	General result
		Peas g.	Oats and bran g.	Milk cc.					
212	30-40 g. soaked peas	30-40	25 (for 38 days)	30-100 (from 28th day)	375 — Slight temporary soreness	130	—	—	Good health throughout: returned to stock
214	30-40 g. "	28	8	310	255 None	128	Bones slightly brittle, rib junctions enlarged, slight haemorrhages, loose teeth	Scurvy	Killed: poor health; slight signs of scurvy
215	30-40 g. "	30	24	0	320 318 Limbs tender	40	Slight haemorrhage in heart muscle, teeth slightly brittle	? Scurvy	Death from unknown cause
88	20 g. "	28	32	0	320 265 Slight soreness of joints	34	Bones fragile, rib junctions ridged, severe haemorrhages	—	Death from severe scurvy
87	20 g. "	19	20	33 (last 32 days)	340 240 Slight soreness of limbs	92	Bones and teeth somewhat brittle	Scurvy	Poor health
208	40 g. "	17.5	51	0	500 Slight soreness	46	Ribs ridged, teeth loose, haemorrhages	—	Death from scurvy
213	30 g. "	18	24	0	358 335 Slight soreness	67	Bones normal, incisors loose, haemorrhages on coecum	? Scurvy	Death from scurvy
91	10 g. "	10	30	0	350 240 Soreness of limbs, loose teeth	21	Bones fragile, severe haemorrhages	—	Death from severe scurvy
92	10 g. "	10	28	0	380 230	28	Bones fragile, severe haemorrhages	Scurvy	Death from severe scurvy
89	20 g. soaked peas re-placed after 38 days by germinated peas	20	33	50 (last 48 days)	330 335 Soreness of joints	38 } 76 }	Normal	Normal	Scurvy cured by germinated peas
90	10 g. soaked peas re-placed after 24 days by 30 g. germinated peas	10	29	55 (last 75 days)	335 270 Soreness of limbs, loose teeth	24 } 84 }	—	—	Returned to stock in good health; scurvy cured by germinated peas

* For 30 days, pea-meal then replaced by tamarind juice for 59 days.

† Here and in the other tables "scurvy" indicates that the rib junctions were not normal, and in most cases they showed the changes which have been previously described as "incipient scurvy." [Tozer, 1918]; as there mentioned, however, it is doubtful whether these alterations indicate anything more than some imperfection in the supply of the accessory substances concerned with growth. They cannot be taken as necessarily indicating scurvy but they are certainly compatible with early or mild scurvy and have to be considered along with the symptoms and other post-mortem signs in estimating the effect of scorbutic diets.

Fig. 1. Weight curves of animals on a diet of ungerminated soaked peas.

A. Control normal weight curve on 30 g. fresh cabbage, oats, bran, and water. B. Curve of No. 348 on average daily ration of 9.6 g. pea-meal, 52 cc. autoclaved milk, and oats and bran *ad libitum*. (Tab. I.) Symptoms of severe scurvy were first noted at S. C. Curve of No. 347 on average daily ration of 8 g. pea-meal, 40 cc. autoclaved milk, oats and bran *ad libitum* for first 30 days. Symptoms of scurvy were first seen at S; after 30 days the pea-meal was replaced by tamarind juice and after 89 days both pea-meal and tamarind juice were given daily. D. Curve of No. 214 on an average daily ration of 28 g. soaked peas: at A.M., after 74 days, an average of 26 cc. autoclaved milk was taken daily. E. Curve of No. 91 on an average daily ration of 10 g. soaked peas (died with severe scurvy). F. Curve of No. 90 on an average daily ration of 10 g. soaked peas. After 24 days (at G.P.) the animal showed all the symptoms of severe scurvy, and the soaked peas were replaced by 10 g. germinated peas, and after 28 days (at *) the ration was increased to 30 g. After 34 days (at I) there was marked improvement, and 60 cc. autoclaved milk was added to the diet: growth at once began and after 78 days (at N.S.) no further symptoms of scurvy could be detected.

extensive haemorrhages in the muscles both recent and of old standing, the bones were fragile and the rib junctions deformed. No. 347 (C, fig. 1) developed swollen and painful joints after 21 days and after 30 days was very lame. Its ration of pea-meal, of which it had eaten about 8 g. daily, was then discontinued and its place taken by a daily ration of 15 to 20 cc. of the juice expressed from dried tamarind, previously swelled in water; the rest of the diet remained as before. It lived 59 days on the tamarind juice without showing much change, further progress of the disease being arrested. It was then given pea-meal in addition, no improvement resulted and the animal was chloroformed after a further period of 15 days. The subsequent post-mortem and histological examinations indicated a condition of chronic scurvy.

It is evident from these results that some small degree of protection from scurvy may be derived from a daily dose of 8 to 10 g. of moistened pea-meal. In comparison with other anti-scorbutic materials it has, however, very little potency. Taking, as is our custom, fresh cabbage leaves as the standard, Delf [1918] has shown that young guinea-pigs can be protected from scurvy and maintained in satisfactory health on a diet of oats, bran, autoclaved milk and 1.5 g. fresh cabbage daily: with 0.5 g. definite symptoms of scurvy appear, varying in intensity in different guinea-pigs. It is evident, therefore, that an average daily ration of 9 g. pea-meal has a protective value less than that of 1.5 g. and about equivalent to that of 0.5 g. fresh cabbage: in other words, cabbage is about 15 times as effective as dry peas. The water content of dry peas is about 12 %, of cabbage about 90 %, so that on the dry weights 8 g. of peas are about equal to 0.05 g. cabbage, a ratio of 160 to 1.

IV. EXPERIMENTS SHOWING THAT PEAS AND LENTILS, AFTER SOAKING IN WATER FOR 24 HOURS, HAVE LITTLE ANTI-SCORBUTIC VALUE.

During the process of soaking, the peas and lentils absorbed roughly their own weight of water and the soaked seeds contained 50 to 55 % water, estimated by drying at 100 to 110°. It was difficult to coax the animals to eat large rations; in one case (No. 212), 30 to 40 g. daily were taken; in general, however, 15 to 20 g. soaked peas and 10 to 15 g. lentils was the limit that was voluntarily consumed. Lentils were eaten without preparation, but it was necessary to skin the peas before the guinea-pigs would eat them; this was done after the ration had been weighed out and involved a loss of about 10 % in weight.

In Table II are given the results of experiments with dry green peas after soaking 24 hours in water at room temperature. Scurvy was prevented when a daily ration of 30 to 40 g. was consumed; with 15 to 30 g., scurvy occurred and although two animals out of six survived the three months of the experiment, they had poor health and made little or no growth. When the ration was reduced to 10 g. death from scurvy occurred as in control animals

Fig. 2. Weight curves of animals on a diet of ungerminated soaked lentils with oats and bran *ad libitum* in each case. 4. Control normal weight curve on 30 g. raw cabbage, oats, bran and water. B. No. 409, on an average ration of 25 g. soaked lentils daily. At * 30 cc., and at * 60 cc. autoclaved milk was added to the diet. C. No. 415 on an average ration of 18 g. soaked lentils. From the first, 30 cc. and after 12 days 60 cc. autoclaved milk was included in the diet. Symptoms of scurvy were first observed after 68 days. D. No. 412 on an average ration of 15 g. soaked lentils. For the first 20 days 30 cc. and afterwards 60 cc. autoclaved milk was given daily. E. No. 413 on an average ration of 10 g. soaked lentils daily. After 4 days 30 cc. and after 26 days 60 cc. autoclaved milk was added to the diet.

(*e.g.* 91 and 92, which died from scurvy in 21 and 28 days). About half the animals received no milk, but the result as regards scurvy was not apparently affected by this.

The case of 89 is interesting. Scurvy, developing on a 20 g. ration of soaked peas, was cured when this ration was substituted by an equivalent weight of germinated peas. Guinea-pig 90 was cured in the same way.

These experiments afford no evidence that soaked peas are better than dry peas. The dry substance of 9 g. dry peas is about equal to that of 18 g. soaked peas, and the effect of the two rations is not markedly different.

Soaked lentils (Table III) have an anti-scorbutic value which is slightly greater than that of soaked peas. Scurvy was prevented with a daily consumption of about 25 g.: with 15 to 18 g. scurvy was apparent but not severe, and 412 and 415 survived the three months of the experiment and put on weight. On a daily consumption of about 9 g., death from severe scurvy occurred in 45 days with much loss of weight. All the animals received autoclaved milk.

In comparing the value of peas and lentils, it should be noted that 10 g. of peas have an average of 30 seeds, and 10 g. of lentils have about 100 seeds.

V. EXPERIMENTS SHOWING THAT GERMINATED PEAS AND LENTILS POSSESS CONSIDERABLE ANTI-SCORBUTIC VALUE.

The peas and lentils after soaking in water for 24 hours were kept moist with access of air (conveniently in a large funnel) for about 48 hours at room temperature. The radicle was then usually about 1 cm. in length [see the figures in the paper by Chick and Hume, 1917]. The guinea-pigs ate the germinated seeds more readily than when they were merely soaked, though they seemed unable to take more than about 15 g. of lentils daily, whether raw or cooked: with peas, 20 to 30 g. were eaten daily for two or three months without ill effect.

Twenty-four animals received varying amounts of germinated peas (Table V). With a daily consumption of 10 g. and over there was satisfactory protection except in the case of one animal (315) out of ten. Out of six animals on a 5 g. ration, two died of some infection: four lived through the three months of the experiment; these grew fairly well and clearly received considerable protection, although they showed some evidence of scurvy when they were killed for examination. On 2.5 g. two died of acute scurvy in 37 and 53 days respectively and though the other two survived to the end of the experiment, both had the disease in a moderate degree. Four animals which ate daily 7 to 10 g. of peas germinated in the dark were also well protected.

With regard to the growth of these animals, it should be noted that most of those which had the larger pea rations were started with peas, oats and bran, but without milk. On this diet little growth was made. The addition

Table III. *Experiments with soaked lentils.*

Number of animal	Ration offered	Average amount consumed daily			Body weight, g.		Symptoms during life	Duration of experiment, days	Post-mortem		Histology of rib junctions	General result
		Lentils g.	Oats and bran g.	Milk cc.	Initial	Final			Normal	Abnormal		
409	30 g. soaked lentils	25	27	39	320	415	Sore limbs	87	Normal	Jaws fragile, haemorrhage in lungs	Normal	Death, diarrhoea, no scurvy
416	30 g. "	23	17	47	345	440	None	54	Normal	Bones normal, slight haemorrhages	Nearly normal	Death from unknown cause, slight scurvy
415	20 g. "	18	19	44	358	480	Soreness of limbs, scurvy position	94	Bones normal, slight haemorrhages		Scurvy	Definite chronic scurvy
412	20 g. "	15	16	48	290	500	Slight soreness of limbs	90	Normal		Nearly normal	Normal
413	10 g. "	10	19	31	362	292	General soreness becoming acute	45	Bones papery, jaws and teeth brittle, slight haemorrhages		Severe scurvy	Death from severe scurvy
414	10 g. "	8	18	33	340	301	Limbs sore, joints swollen	45	Bones soft, knees fractured and swollen, haemorrhages severe		Severe scurvy	Death from severe scurvy

Table IV. *Experiment with germinated lentils.*

404	10 to 20 g. germ. lentils	12.2	18	47 (after 30 days)	330	438	None	90	Normal		Normal	Complete protection
405	10 "	7.7	39	50 (after 30 days)	358	640	"	91	"		"	"
407	10 "	9.5	28	50 (after 30 days)	330	620	"	93	"		"	"
411	10 "	15.1	16	46 (after 30 days)	320	570	"	94	"		"	"
427	5 g. "	5	26	57	302	540	"	90	Bones brittle		Severe chronic scurvy	Chronic scurvy, partial protection
428 A	5 g. "	5	49	42	342	465	"	90	Normal		Chronic scurvy	Protection, signs of scurvy in histology only
829	5 g. "	5	17	56	320	275	General soreness	51	Fragile bones, severe haemorrhages		Severe scurvy	Death from severe scurvy
830	5 g. "	5	25	57	333	355	Knees much swollen	73	do.		"	Death from scurvy
423	2.5 g. "	2.5	35	47	318	330	Knees sore	74	Brittle bones and teeth, haemorrhage in stomach, signs of infection		Scurvy	Death from infection, scurvy also
430	2.5 g. "	2.5	27	55	310	362	General soreness	78	Bones and teeth brittle, haemorrhage in one knee and coccyx, signs of infection		Severe scurvy	do.
431	2.5 g. "	2.5	62	51	330	375	Limbs sore	70	Teeth brittle, two rib junctions ridged, signs of infection		Scurvy	do.
817	2.5 g. "	2.5	30	56	370	393	Limbs sore, very lame	90	Bones hard, knees swollen, severe haemorrhages, 3 rib junctions ridged		"	Severe chronic scurvy
839	2.5 g. "	2.5	15	57	345	337	Lame, limbs sore	92	Bones very fragile, rib junctions ridged, severe haemorrhage		Chronic scurvy	"
818	2.5 g. "	2.5	28	54	348	355	Lame, scurvy position	48	Bones fragile, haemorrhages, pneumonia		? Scurvy	Death, from bronchopneumonia, scurvy also

of autoclaved milk at once initiated growth (312, Fig. 3, *B*): autoclaved skimmed milk acted in the same way though less effectively (308, Fig. 3, *C*): with tinned milk (315, Fig. 3, *D*) there was a short period of improvement but the animal afterwards lost weight, refused food and died with diarrhoea.

These scanty data suggest that germinated peas are deficient in the fat-soluble growth factor of which whole milk seems to be the best source among

Fig. 3. Weight curves of animals on a diet of germinated peas.

A. Normal weight curve on 30 g. fresh cabbage, oats, bran and water. B. No. 312 on an average daily ration of 20-30 g. germinated peas. After 38 days (at A.M.) 60 cc. autoclaved milk was added to the diet. C. No. 308 on a daily ration of 20 g. germinated peas. After 62 days (at A.S.M.) autoclaved skimmed milk was added to the diet. D. No. 315 on an average daily ration of 30 to 12 g. germinating peas. After 44 days (at T.M.) tinned milk was added to the diet. E. No. 310 on average daily ration of 20 g. germinated peas. After 44 days (at A.M.) autoclaved milk was added to the diet. F. No. 337 on a daily ration of 10 g. germinated peas. After 38 days (at A.M.) autoclaved milk was added to the diet. G. No. 437 on a daily ration of 2.5 g. germinated peas with 60 cc. autoclaved milk from the first. After 30 days, at S, symptoms of scurvy first developed.

the different types of milk tried. Before this conclusion could be accepted as an established fact, it would be necessary to show that the diet was adequate as regards all *other* components, to a deficiency of which growth limitation could be attributed—such as the nature and quantity of (1) protein, (2) mineral salts, and (3) water-soluble growth factor. No. 3 is provided in abundance in a diet consisting of peas, oats, and wheaten bran, and it is probable that a possible defect in any of these constituents as regards (1) or (2) will be made good by admixture with the others. The fact that in all these experiments without milk, weight was satisfactorily *maintained* is in favour of this conclusion. It is unfortunate that the point could not be quickly settled by observing whether normal growth takes place on addition to the diet of a small proportion of butter fat, cod liver oil, or other source of the fat-soluble growth factor—the guinea-pig being unable to tolerate pure fat given in this form.

In case of the animals with the smaller rations of peas, 60 cc. of autoclaved milk was offered daily throughout the experiment and was as a rule well taken; in the absence of severe scurvy growth was made (Fig. 3, compare *F* and *G*). It seems, therefore, that a daily ration of 10 g. germinated peas with milk will prevent scurvy and assure good health and growth. The cure of two animals from scurvy by germinated peas has already been mentioned (see Table II).

With germinated lentils (Table IV) complete protection was obtained with a daily consumption of about 10 g. and partial protection with 5 g., while with 2.5 g. definite symptoms developed in every case. In all cases life was substantially prolonged, even when scurvy was not prevented.

With 10 g. of lentils no growth was made without milk but when autoclaved milk was added to the oats and bran, the weight rapidly increased (Fig. 4, *B*, after 30 days). The 2.5 and 5 g. series had milk from the start and made good growth until symptoms of scurvy appeared. The history of the guinea-pigs with the smallest lentil rations was, however, unfortunately obscured by four of the six animals dying of intercurrent disease as is often the case when the diet contains only minimal amounts of an essential accessory factor.

The results with germinated lentils are thus in accord with those for peas: in both cases a 5 g. daily ration seems to be just below, and 10 g. well above, the minimum required for protection.

In Table VI are summarised the results of the foregoing experiments with peas and lentils. In the dry condition peas show traces of anti-scorbutic properties as evidenced by prolongation of life although scurvy is not prevented; the same result was obtained by Fürst [1912]. When soaked in water for 24 hours at room temperature scurvy was prevented with a large daily ration, 30 to 40 g. peas (dry weight, 15 to 20 g.) or 20 to 30 g. lentils (dry weight, 10 to 15 g.). The data do not permit one to decide whether any anti-scorbutic virtue is created during the process of soaking in water, for in the case of the

Table V. *Experiment with germinated peas.*

Number of animal	Ration offered	Average amount consumed daily			Milk cc.	Body weight		Symptoms during life	Duration of experiment, days	Post-mortem	Histology of rib junctions	General result
		Peas g.	Oats and bran g.	Peas g.		Initial	Final					
312	30 g. germinated peas	25	28	50 (after 36 days)	50	330	620	All the animals on 10 g. peas and upwards shewed signs of soreness of limbs during life unaccompanied, however, by symptoms of scurvy, excepting in one case (No. 315)	93	Normal	Normal	Protection, no scurvy
323	30 g.	23	25	40 (after 26 days)	40	410	51		83	—	—	Good health, returned to stock do.
314	30 g.	21	25	40 (after 29 days)	40	270	515		88	—	—	do.
315	30 g.	30 to 12	24	47 (after 44 days)	47	260	270		75	Fragile bones, loose teeth, muscular and intestinal hæmorrhages	—	Death from diarrhoea, scurvy also
308	20 g.	20	28	50 (after 60 days)	50	400	495		93	—	—	Good health, returned to stock
309	20 g.	18	29	52 (after 38 days)	52	305	550		93	Normal	Normal	Protection, no scurvy
325	15 g.	14.5	25	48	48	420	600		84	—	—	Good health, returned to stock
311	10 g.	10	28	52	52	320	515		94	Rib junctions slightly ridged	Normal	Protection, no scurvy
313	10 g.	10	30	47	47	290	392		69	Bones somewhat fragile, hæmorrhages only in ileum and duodenum	Normal	Died from unknown cause, ? any scurvy
310	10 g.	10	27	52 (after 38 days)	52	320	535		93	Rib junctions slightly ridged	? Scurvy	Protection
327	5 g.	5	37	53	53	345	525	Slight soreness persistent	94	Rib junctions slightly thickened	Severe scurvy	Scurvy
328	5 g.	4.1	25 (after 68 days)	39 (after 68 days)	39	305	435	Slight soreness persistent	87	Rib junctions slightly ridged, muscles very dark	? Scurvy	Protection
337	5 g.	5	26	45	45	345	560	Scurvy position	97	Rib junctions slightly enlarged, slight hæmorrhages	Scurvy	Slight scurvy
341	5 g.	5	28	44	44	350	565	Slight soreness	92	do.	Normal	Slight scurvy
434	5 g.	5	29	45	45	330	360	None	29	Slight subcutaneous hæmorrhage	Severe scurvy	Death from infection, also scurvy
435	5 g.	5	47	55	55	330	378	Soreness persistent	62	Spleen diseased	do.	do.
436	2.5 g.	2.5	34	46	46	343	272	Loss in weight	53	Bones very fragile, severe hæmorrhages	do.	Death from severe scurvy
437	2.5 g.	2.5	35	59	59	309	433	Joints swollen and tender	94	Muscular hæmorrhages partly absorbed	Scurvy	Chronic scurvy
438	2.5 g.	2.3	22	51	51	330	250	Soreness, scurvy position	37	Bones very fragile, teeth loose and brittle, hæmorrhages	Scurvy	Death from severe scurvy
439	2.5 g.	2.5	46	56	56	325	645	Soreness lasting 32 days	90	Rib junctions slightly enlarged, teeth loose, jaw brittle	? Scurvy	Slight scurvy
342	10 g. peas germinated in darkness	7	22	57	57	328	515	Slight soreness	94	Normal	Normal	Protection
343	10 g. do.	10	24	52	52	340	642	Slight soreness lasting 35 days	102	—	—	Good health, returned to stock
344	10 g. do.	9.5	21	51	51	313	480	Scurvy position	94	No evidence of scurvy	Normal	Protection
345	10 g. do.	9	28	49	49	340	450	None	58	do.	? Scurvy	Death from intestinal obstruction

— = not examined.

pea-meal it was not possible to induce the animals to take a ration larger than 10 g. (dry weight, 9 g.), but the change, if any, cannot be large.

After germination, however, the story is quite otherwise; scurvy was prevented successfully in case of both lentils and peas with a daily ration of 10 g. (dry weight, 5 g.); in many cases 5 g. daily (dry weight, 2.5 g.) was found to be sufficient, while with 2.5 g. daily (dry weight, 1.2 g.) some degree of protection was obtained.

Table VI.

Anti-scorbutic material	Daily ration, g.	Water content, % (approx.)	Dry weight, g.	No. of animals	Time of experiment, days	Result
Peas						
Pea-meal, moistened	8-10	12	7-8.8	3	31-105	Scurvy, but some degree of protection
Soaked 24 hours ...	30-40	50	15-20	2*	128-130	No scurvy
	20	50	10	4	34-114	Scurvy, but distinct protection
	10	50	5	2	21-28	Death from scurvy
Germinated ...	20-30	50	10-15	6	75-93	Good health in 5 cases
	10	50	5	3	69-94	No scurvy
	5	50	2.5	5†	87-97	Signs of scurvy, but good health in 4 cases
	2.5	50	1.2	4	37-94	Scurvy in all cases, but some degree of protection
Lentils						
Soaked 24 hours ...	30	50	15	2	54-87	No scurvy, protection
	20	50	10	2	90-94	Signs of scurvy but considerable protection
	10	50	5	2	45	Death from scurvy
Germinated ...	10	50	5	4	91-94	No scurvy, protection
	5	50	2.5	4	51-90	Scurvy in 2 cases, but considerable degree of protection
	2.5	50	1.2	6	48-90	Scurvy, but considerable protection
Fresh lemon juice ...	1.5 cc.	—	—	6	74-90	No scurvy, protection
Fresh orange juice ...	1.5 cc.	—	—	5	62-91	" "
Fresh cabbage leaves	1.5 g.	90	—	5	90	" "
Fresh swede juice ...	2.5 cc.	—	—	8	36-90	" "
Fresh carrot juice ...	20-30 cc.	—	—	8	27-94	" "
Fresh beetroot juice ...	20 cc.	—	—	3	47-90	Scurvy
Fresh green beans ...	5 g.	90	—	4	—	Protection, no scurvy
Control A† ...	—	—	—	4	23-30	Death from scurvy
Control B† ...	—	—	—	4	34-40	" "

* Omitting No. 215 which died from other causes.

† Omitting 434 and 435 which suffered from an infection.

‡ In case of A the diet consisted of oats, bran and water only; in case of B autoclaved milk 60 cc. was added to the diet.

The anti-scorbutic value of the soaked seeds is thus seen to be increased 3- to 6-fold after 48 hours of germination at room temperature and becomes comparable with that of many fresh vegetables. While distinctly less than that of raw cabbage (minimum protective dose for guinea-pigs 1.5 g. [Delf, 1918]), swede (minimum protective ration 2.5 g. [Chick and Rhodes, 1918]), or fresh orange or lemon juice (minimum protective dose 1.5 cc. [see Chick, Hume, Skelton and Smith 1918]), it is equal to that of raw green (runner)

VI. EXPERIMENTS WITH COOKED GERMINATED LENTILS.

Animals were selected which ate raw lentils with relish and amongst these a further selection was made of those which would eat them readily after cooking. It was found (Table VII) that a ration of 10 g. boiled for half or one hour in water was insufficient to protect from severe scurvy. The animals did well for the first two weeks, they put on weight (Fig. 5, *D*), but soon suffered from swollen and sore joints and, when chloroformed, were all found to be suffering from severe scurvy. These animals all received a milk ration.

Five animals were next given a larger ration, 15 g., boiled in water for only 15 minutes, but it was found impossible to ensure that the whole of the amount was eaten. In all cases life was prolonged. One animal, 852 (Fig. 5, *C*), ate an average of 14 g. daily for 91 days and received considerable protection, but even this case showed swollen knees and lameness during life. The remaining four animals were all severely crippled after 30 to 40 days. Cooking had, therefore, reduced the anti-scorbutic value of about 12 g. of germinated lentils to rather less than that of 2.5 g. of the raw material (see Table IV), *i.e.* a loss of about 75 %. This result is in accord with that already established in the case of fresh cabbage, which has been shown to lose about 70 % of its antiscorbutic value when steamed for 20 minutes at 100° [Delf, 1918].

Major E. D. W. Greig, I.M.S., has suggested that the loss during boiling could be reduced by adding traces of some acid to the cooking water. Small amounts of citric acid have no harmful effects on young guinea-pigs [Delf, 1918]. With cabbage, citric acid accelerates the destruction of antiscorbutic stuff by boiling, and with germinated lentils very similar results have been obtained. The cell-sap of the growing lentils is slightly alkaline to litmus and remains neutral or slightly alkaline after boiling with a 0.005 % solution of citric acid. When the concentration is increased to 0.5 %, however, the washed seeds yield a juice which is distinctly acid to litmus. These two solutions were used respectively as the cooking water for the rations in two sets of experiments (Table VIII), and the results show that there is certainly no advantage, and possibly some disadvantage, in using acidified instead of ordinary tap water.

VII. WILTSHIRE'S EXPERIMENTS ON THE VALUE OF GERMINATED BEANS IN THE TREATMENT OF HUMAN SCURVY.

Acting on the suggestion made in the preliminary statement of these results [Chick and Hume, 1917, p. 157], Major H. W. Wiltshire, R.A.M.C., has made a most interesting trial of germinated beans in the treatment of scurvy in Serbian soldiers [1918]. Peas could not be obtained, the available lentils were decorticated and would not germinate, so "haricot beans were...first soaked in clean water for 24 hours, and then placed in tin trays for 48 hours to germinate. Old ration biscuit tins, cut in half longitudinally

Table VII. *Experiment with cooked germinated lentils.*

Number of animal	Ration offered	Average amount consumed daily			Body weight g.		Symptoms during life	Duration of experiment, days	Post-mortem	Histology of rib junctions	General result
		Lentils g.	Oats and bran g.	Milk cc.	Initial	Final					
809	10 g. germ. lentils boiled 1 hr in water	9.4	28	54	320	260	Scurvy position, great soreness	25	Bones fragile, haemorrhages	Severe scurvy	Severe scurvy
810	do.	8.3	10	47	325	300	do.	30	"	"	"
811	do.	10.3	15	53	350	353	Scurvy position, lame	30	"	"	"
812	do.	8.2	14	55	322	288	do.	29	"	"	"
819	10 g. germ. lentils boiled 1 hr. in water	9.2	13	44	340	275	Soreness, scurvy position	21	"	"	"
820	do.	6.6	15	47	335	275	do.	26	"	"	"
821	do.	8.5	13	56	340	280	do.	37	"	"	"
822	do.	10.2	17	55	325	400	do.	27	Not examined	Not examined	Severe scurvy, cured with orange juice
846	15 g. germ. lentils boiled 15 min. in water	12.3	12	59	370	350	Sore and much swollen knees, scurvy position	82	Bones fragile, severe haemorrhages	Severe scurvy	Emaciated, lame, partial protection
849	do.	12.1	10	52	325	285	do.	53	do.	"	Death from severe scurvy
850	do.	6.3	11	50	325	235	do.	52	do.	Severe scurvy	"
851	do.	13.7	13	55	350	315	do.	60	do.	? Scurvy	"
852	do.	14.2	22	59	360	588	do.	91	Bones firm, old haemorrhages at knees, slight recent haemorrhage	Partial protection	Partial protection

Table VIII. *Experiment with germinated lentils cooked with citric acid.*

859	15 g. germ. lentils boiled 15 min. in 0.005 % citric acid	12.9	15	59	325	555	Soreness, knees swollen	94	Bones normal, slight muscular haemorrhages about knees	? Scurvy	Slight scurvy
860	do.	13.3	8.5	53	330	300	do.	60	Bones soft, loose teeth, severe haemorrhages	Severe scurvy	Death from severe scurvy
872	do.	12.5	9	57	352	382	None	38	Bones slightly fragile, ribs slightly ridged	Scurvy	Death from cause unknown
873	do.	9.4	6	52	331	270	Knees swollen	36	Bones papery, loose teeth, severe haemorrhages and in one lung	Severe scurvy	Death from severe scurvy
863	15 g. germ. lentils boiled 15 min. in 0.5 % citric acid	11.7	9	55	337	244	Scurvy position	30	Bones fragile, haemorrhages severe	"	"
864	do.	12.9	9	57	333	300	Sore, knees swollen, scurvy position	89	Bones fragile, rib-junctions nodular, severe haemorrhages	Severe scurvy	Chronic scurvy
874	do.	15	7	58	320	345	Sore, swollen knees	90	Bones fragile, rib junctions ridged, two ribs broken, severe haemorrhages	"	"
875	do.	13.3	5	56	335	270	Sore, swollen knees, became helpless	59	Bones very fragile, rib junctions much swollen, severe haemorrhages	"	Death from severe scurvy

and freely perforated with holes, were found serviceable for this purpose. They were easy to make, clean to handle, and each half held seven pounds of beans, a day's dose for 28 patients. Since germination takes about 48 hours at 60° F., it can easily be carried out in this country (Serbia) in May, when the mean temperature is 67° F. The whole process is very simple, the only essentials being that the seeds must be kept moist but not shut off from

Fig. 5. Weight curves of animals on a diet of cooked germinated lentils, oats, bran and autoclaved milk. A. Control normal weight curve on 30 g. raw cabbage, oats, bran and water. B. Mean weight curve of 5 animals on diet of 10-15 g. germinated lentils boiled 15 min. in water, oats, bran and 60 cc. autoclaved milk daily: at † No. 852 died. C. Weight curve of No. 852, the only animal of this set which escaped scurvy. D. Mean weight curve of three animals (819, 820, 821) on diet of 7-9 g. germinated lentils boiled half an hour in water, oats, bran and autoclaved milk. E. Mean weight curve of Nos. 864, 874, the two best cases on diet of 13-15 g. germinated lentils boiled 15 min. in solution of 0.5 % citric acid. At S, definite symptoms of scurvy were first seen. F. Mean weight curve of Nos. 859, 860, the two best cases on diet of 10-15 g. germinated lentils cooked 15 min. in solution of 0.005 % citric acid. At † No. 860 died.

free circulation of air. After germination the ten minutes' boiling was ample to fit the beans for eating.

"Two wards, each containing 27 beds, were specially devoted to the purpose of comparing the therapeutic values of these germinated beans and fresh lemon juice. Scurvy patients were admitted to these two wards alternately, without selection. In one ward each patient was given four ounces of fresh lemon juice daily, the juice being expressed in the ward, sweetened slightly and given as a measured dose by the sister. In the other ward each patient received a portion of germinated beans which had weighed four ounces in the dry state.

"Thirty patients were treated with lemon juice and twenty-seven with beans. Contrasting them as regards severity of disease at the commencement of treatment, those treated with beans were, on the whole, slightly worse. The difference would be too small to mention, save that it justifies the definite statement that the bean cases were certainly as severe as the lemon cases. Comparing the results of treatment there is a small but definite difference in favour of those treated with beans, 70 % being cured within four weeks as against 53 % of those treated with lemon juice. These figures favour the bean cases unduly, the real difference being better expressed by the (average) time taken for the gums to return to normal, which was 3.1 weeks for bean cases and 3.4 weeks for lemon cases."

Evidently germinated beans are satisfactory for treatment. Whether they are as efficient as fresh lemon juice cannot of course be gauged without further experience with different doses. Experiments on guinea-pigs show (Table VI above) that, as a preventive, lemon juice (but not lime juice: Chick, Hume, Skelton and Smith, *Lancet*, November 30th, 1918) is a good deal superior to germinated peas or lentils.

It is, however, as a *preventive* of scurvy that the inclusion of germinated pulses in a human diet deficient in fresh fruit and vegetables, is principally to be recommended, and so far no definite trials of this type have been reported. In many parts of the world there exists the practice of eating certain seeds in the germinated condition, although there is no suggestion that the anti-scorbutic value of such foods has been appreciated. In the Dutch Indies and Federated Malay States germinated beans or "tow-gay" are eaten raw as a common article of diet [Grijns, 1901; Private communication, Brig.-Gen. Anderson]. In certain districts of China it is the custom to take part of the rice in the germinated condition and especially in the north, beans are artificially sprouted for food in the winter [Report, 1885].

There is little doubt that the anti-scorbutic value of certain fermented beverages, firmly believed in by our ancestors, owed their virtue to the germinated grains from which they were prepared. Captain Cook¹ was a firm believer in the anti-scorbutic properties of a fresh infusion of malt, "sweet-wort." He always took large quantities of malt to sea with him and

¹ *Captain Cook's Voyages of Discovery.*

the sweet-wort was served out to his men in large quantities when there was any risk of scurvy. "Small beer" also enjoyed a great reputation and in many Arctic expeditions in the first half of the nineteenth century, malt, yeast, and a brewing plant were included in the equipment [see A. Henderson Smith, 1918].

Modern beer prepared from "high dried" malt has been shown by Harden and Zilva [1918] to possess no appreciable anti-scorbutic value and there is no doubt that the virtue these more ancient beverages possessed was due to the freshness of the product, the drink being prepared quickly from freshly germinated grain and consumed shortly after preparation. An interesting incident illustrating this point was reported during the present war by Dyke [1918], in connection with an outbreak of scurvy among a Kaffir labour battalion in France. The diet of this community was arranged to produce as nearly as possible that of the native kraals, where, in spite of a limited diet, scurvy is unknown. The arrangements even included the provision of a native beer brewed from millet, of which large quantities are regularly consumed in Africa. In the enquiry which followed the outbreak of scurvy, the following fact emerged, viz. that the beer as brewed in South Africa was prepared from germinated millet, but that in France the process of germination had been omitted for reasons of convenience.

SUMMARY.

1. Fürst's observation that the content of anti-scorbutic accessory factor in dry peas and lentils is much increased on germination has been confirmed.

2. The antiscorbutic value of these seeds after soaking in water for 24 hours, and germination for 48 hours at room temperature is 5- to 6-fold that of the dry seeds; while inferior to that of orange and lemon juice or cabbages and swedes, it is equal to that of many other vegetables such as green (runner) beans or potatoes, and superior to that of carrot or beetroot. Table VI contains a summary of these results.

3. These seeds, whether in the dry or germinated condition, do not, however, contain a sufficient amount of the growth-promoting substances to induce satisfactory growth in the experimental animals (guinea-pigs) in the absence of milk from the diet. It seems probable that it is the fat-soluble factor that is deficient.

4. A considerable proportion of the anti-scurvy power generated in these germinated seeds is destroyed by boiling: cooking of these germinated seeds should therefore be as short as possible.

5. Wiltshire has shown that germinated beans can be used with success in the treatment of human scurvy. Other evidence is quoted from experience of human diets showing that germinated seeds and their products form valuable material for the prevention of human scurvy.

REFERENCES.

- Chick and Hume (1917). *Trans. Soc. Trop. Med. Hyg.*, **10**, 141.
Chick, Hume and Skelton (1918). *Biochem. J.*, **12**, 131.
Chick, Hume, Skelton and Smith (1918). *Lancet*, Nov. 30th.
Chick and Rhodes (1918). *Lancet*, Dec. 7th.
Dyke (1918). *Lancet*, Oct. 19th, p. 513.
Delf (1918). *Biochem. J.*, **12**, 448.
Fürst (1912). *Zeitsch. Hyg.*, **72**, 121.
Greig (1917). *Indian J. Med. Research*, **4**, 818.
Grijns (1901). *Geneesk. Tijdschrift Med. Ind.*, **1**.
Harden and Zilva (1918). *J. Inst. Brewing*, **24**, 197.
Holst and Frölich (1912). *Zeitsch. Hyg.*, **72**, 1.
Mesopotamia Commission Report (1917), **10**, 71.
Report on Public Health in China (1885).
Smith (1918). *Lancet*, Dec. 14.
Tozer (1918). *Biochem. J.*, **12**, 445, and Plate II.
Wiltshire (1918). *Lancet*, Dec. 14.