CLXXXI. METABOLISM IN SCURVY. II.

THE NITROGEN ABSORPTION AND RETENTION OF GUINEA-PIGS.

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THE object of this investigation was to ascertain whether the pathological changes in the guinea-pig which produce the syndrome of scurvy are connected with a disturbance in the absorption or in the retention of nitrogen. When a healthy guinea-pig of about 300 g. is placed on a scorbutic diet well balanced in other respects and is kept under hygienic and comfortable conditions it will grow and consume its normal requirements for about 21 days or even longer, although it has been suffering from scurvy for some days. After this stage the appetite diminishes and loss in weight is recorded owing to the aggravated condition of the animal caused by the progress of the disease. In the case of guinea-pigs kept in metabolism cages the diminished consumption of food may manifest itself a few days earlier owing to the greater discomfort caused by the scorbutic condition in the exertion of reaching the food. Animals maintained on scorbutic diets which are also deficient in other respects, such as a one-sided oats diet or a balanced mixture composed mainly of constituents which are not natural for the guinea-pig and consequently not very palatable, will begin to consume less of their food quite early. It is evident, therefore, that the character of the diet is of great importance in dietetic experiments in which the bearing of scurvy on metabolism is studied. The diet used in this investigation has proved to be suitable for this purpose. Despite the less comfortable conditions of the metabolism cage as compared with the ordinary cage, the animals began showing a tendency to consume less food at a time when the early symptoms of scurvy are usually in evidence and consequently any disturbance in the absorption and retention of nitrogen associated with the onset of scurvy, or the development of the disease previous to the manifestation of obvious symptoms, would have been reflected at a time when the consumption of food was normal. Our results suggest that such disturbance does not take place. Whether a deranged metabolism of the

absorbed nitrogen is caused by the vitamin deficiency is being studied, alongside similar problems with a view of throwing some light on the mode of action of the antiscorbutic factor.

Experimental procedure.

Young growing guinea-pigs were employed since the quickly developing animal was considered to be more sensitive in manifesting a change in its nitrogen balance. The animals were kept in modified Hopkins metabolism cages, in a room the temperature of which never fell below 65° F. The cages which were 25 cm. in diameter were fitted with a detachable zinc floor perforated with 2 cm. holes, thus enabling the faeces to drop out of the cage fairly freely. At the end of the tunnel which formed an arm of the cage there was a frame in which a shutter, separating the cage from the food receptacle, was introduced. In the centre of the shutter was a round hole of such a size as to permit the animal free access to the food but preventing it from dragging any food into the tunnel or the cage. A glass separator, described by Gross and Connell [1923], was attached to the collecting funnel. Before the commencement of an experiment several specially chosen guinea-pigs of suitable weight were placed separately in the metabolism cages and were watched for some time. Only those animals which showed the capacity of adapting themselves without much trouble to this existence were selected for investigation.

The food mixture was made up as follows. 325 g. of bran were thoroughly mixed with 190 g. of barley meal and enough warm water was added to the mixture to make it damp and give it a convenient consistency. 280 g. of middlings and 180 g. of fish meal were then added and the whole mixture was rubbed in small quantities between the hands in order to ensure a uniform distribution. Each batch of food, which was prepared for several days, was analysed for its nitrogen and dry matter content and was kept in an air-tight tin receptacle in the cold room. Bi-weekly nitrogen and dry matter determinations have shown that the composition of each batch of food remained constant. Besides this food mixture, which was given *ad lib.*, the animal received a definite quantity of milk every day, delivered by hand, so that the quantity of liquid administered per day was always the same. The milk was made up from an analysed dried full-cream milk stock powder which was kept under the same conditions as the food mixture and which, by frequent analyses, was shown to keep a constant composition. 31.250 g. of this powder (containing 97.15 % of dry matter) was dissolved in 250 cc. of water and kept in a well-stoppered bottle in the cold room. The uniformity of the nitrogen content was assured by analysing each batch of milk. 40 cc. of milk was administered to the animal daily in 10 cc. doses. On Sundays two doses of 10 cc. each of twice the normal strength of milk were given. 30 g. of the food mixture was weighed out every morning into a tared glass vessel which was placed in the food compartment. The following morning at the same time the food was weighed back. After careful observation we have satisfied ourselves that no

visible particle of food ever penetrated the tunnel or cage during the course of the experiments. The dry matter and nitrogen content of the residual food mixture were determined as soon as possible after collection.

The urine was collected every morning at the same time in a weighed flask containing a known quantity of nitrogen-free acid—tartaric acid in the case of guinea-pig No. 1 and phosphoric acid in the remaining animals—and was weighed immediately after collection. The receptacles in which the faeces were received had cardboard covers which were kept damp in order to check evaporation as much as possible. After ascertaining the wet weight of the faeces the material was dried in an oven at 100° to brittle consistency, when it was ground and aliquot portions were taken for the determination of dry matter and of nitrogen.

The dry matter content of the food, oven-dried faeces, and evaporated urine was determined by drying the material at 78° in a vacuum over phosphorus pentoxide to constant weight. The nitrogen content was determined by a micro-method in which the material was oxidised with boiling concentrated sulphuric acid in the presence of anhydrous potassium sulphate and copper sulphate and the resulting ammonia after addition of sodium hydroxide was aspirated into standard acid. 0.5 g. was employed in the case of the food mixture and of the oven-dried faeces and 1.0 g. of the urine as collected. The carbon was determined on the vacuum-dried urine by the usual combustion method, 0.1 g. of material being used in the determination.

Discussion of results.

Guinea-pig No. 1 (Tables I and IV). The experiment on this animal was divided into four periods. The first period was devoted to the investigation of the suitability of the technique. It commenced on Feb. 1, 1928 and lasted 28 days. During this period the animal received the basal diet *ad lib.*, 40 cc. of milk and 5 cc. decitrated lemon juice *per diem*. The antiscorbutic dose contained about 0.5 g. of solids, mostly sugar. The nitrogen administered in this way was less than 1 mg. per day and was consequently outside the limits of accuracy of the method. The quantity of the food mixture consumed by the animal was of the same magnitude as that eaten by a similar animal in an ordinary cage, which with the help of the above antiscorbutic dose was found by one of us to promote normal growth and excellent health in guinea-pigs of this age. The adequacy of this basal diet for the guinea-pig is, therefore, apparent. During the 28 days of this period the animal gained about 50 g.—a gain which is, perhaps, rather less than the normal but which may be considered quite satisfactory in view of the lesser comfort of the metabolism cage. It will be seen that the nitrogen balance during this period was definitely positive and more or less commensurate with the growth. The nitrogen intake (average 614 mg.) varied roughly from 500 to 700 mg. per day and was controlled by the amount of the basal mixture consumed daily and by the variation in the nitrogen content of the different batches of this mixture. The average daily

Table I.

Date	Weight (g.)	Dry food (g.)	Food-N (mg.)	Milk-N (mg.)	Total N intake (mg.)	Weight of urine (g.)	Urine-N (mg.)	Weight of faeces (g.)	Dry weight of faeces (g.)	% of dry matter in faeces	Faecal N (mg.)	Faecal N % of total N intake	Total N output (mg.)	Nitrogen balance (mg.)
ii. 1	305	15.52	600.7	84	684.7	26.233	449	6.202	4.462	71.9	98	14.3	557	+138
2	310	14.65	564.7	84	648.7	32.300	510	6.579	4.159	63.2	104	16.0	614	+35
3	310	15.64	615.0	84	699.0	25.152	443	10.021	4.128	41.2	100	14.3	543	+166
4	315	14.72	421.6	84	505.6	23.345	293	12.188	6.186	50.7	149	29.4	442	+64
5	315	16.66	453.6	84	537.6	23.305	378	3.304	1.942	58.8	50	9.3	428	+110
6	310	15.54	431.0	84	515.0	20.471	376	10.138	5.216	50.5	150	29.1	526	-11
7	320	18.09	510.8	84	594.8	32.330	394	12.960	4.810	37.2	125	21.0	519	+76
8	322	16.01	444.8	84	528.8	34.957	435	5.096	4.455	89.4	102	19.3	537	-8
9	325	18.07	637.0	84	721.0	26.286	423	9.078	4.373	45.9	97	13.5	520	+201
10	325	16.42	567.2	84	651.2	28.194	458	12.485	5.580	44.6	137	21.0	595	+56
11	327	13.84	487.3	84	571.3	18.840	476	8.105	4.179	51.5	95	16.6	571	0
12	322	9.88	373.8	84	457.8	22.618	606	8.545	4.568	53.4	86	18.8	692	-235
13	320	14.85	522.6	84	606.6	27.060	592	7.128	4.032	56.5	92	15.2	684	-77
14	332	16.84	601.0	84	685.0	25.493	381	9.210	4.434	48.1	103	15.0	484	+201
15	340	17.87	642.2	84	726.2	26.170	428	12.020	4.187	34.8	99	13.6	527	+199
16	345	17.68	620.2	84	704.2	18.482	319	12.157	4.103	33.7	92	13.1	411	+293
17	347	16.82	590.7	84	674.7	22.820	370	10.099	5.099	42.7	114	16.9	484	+191
18	350	13.60	476.2	84	560.2	25.469	436	11.920	4.553	45.1	108	19.3	544	+16
19	345	13.50	473.2	84	557.2	16.630	542	12.700	6.359	50.1	140	25.1	682	-125
20	335	16.71	538.2	84	622.2	19.022	579	8.819	4.335	49.1	107	17.2	686	-64
21	342	19.01	607.4	84	691.4	21.541	497	7.902	4.006	50.7	89	12.9	586	+105
22	360	20.21	694.8	84	778.8	29.985	488	6.492	3.606	55.5	86	11.0	574	+205
23	360	19.73	660.5	84	744.5	35.361	597	13.082	5.553	42.3	128	17.2	725	+19
24	360	19.89	674.5	84	758.5	27.229	539	11.424	4.937	43.2	117	14.9	656	+102
25	355	18.14	570.8	84	654.8	25.271	403	6.053	3.747	61.9	90	13.7	493	+162
26	350	10.84	339.5	84	423.5	14.345	379	10.652	5.521	51.8	127	29.9	506	-83
27	340	14.28	458.3	84	542.3	20.674	498	11.597	6.352	54.7	158	29.1	656	-114
28	355	8.92	308.7	84	392.7	21.126	250	8.331	3.682	44.2	84	21.4	334	+59
29	360	9.45	329.6	42	371.6	26.496	288	6.080	2.297	37.7	51	13.7	339	+33

iii.

1	345	9.84	337.9	42	379.9	14.712	310	5.445	2.297	42.1	53	13.9	363	+ 16
2	342	10.00	340.0	42	382.0	20.538	318	5.666	2.480	43.7	58	15.1	376	+ 6
3	350	10.34	356.0	21	377.0	16.315	447	6.884	3.222	46.8	77	20.4	524	- 147
4	335	10.31	354.0	21	375.0	27.603	491	7.412	3.052	41.2	55	14.7	546	- 171
5	325	10.32	355.3	42	397.3	24.512	432	7.511	2.895	38.5	64	16.1	496	- 99
6	325	7.50	258.4	21	279.4	18.375	333	7.194	2.831	39.3	79	28.3	412	- 133
7	320	7.51	257.6	21	278.6	17.175	314	7.373	3.389	45.9	79	28.3	393	- 114
8	315	19.94	712.8	84	796.8	27.388	537	12.442	5.232	42.1	119	14.9	656	+ 141
9	315	18.73	669.2	84	753.2	27.715	506	9.118	5.479	60.1	132	17.5	638	+ 115
10	320	15.24	537.2	84	621.2	25.119	491	8.230	4.109	49.9	97	15.6	588	+ 33
11	320	13.15	461.5	84	545.5	15.052	366	9.516	5.835	61.3	142	26.0	508	+ 37
12	315	17.92	625.6	84	709.6	27.123	648	7.647	5.322	69.5	124	17.4	772	- 72
13	320	16.01	564.2	84	648.2	29.226	406	6.492	3.309	50.9	77	11.9	483	+ 165
14	325	12.89	458.9	84	542.9	27.806	260	7.590	4.440	58.5	104	19.2	364	+ 179
15	325	18.20	655.7	84	739.7	24.102	459	6.119	4.431	72.4	102	13.8	561	+ 179
16	332	16.72	603.8	84	687.8	23.583	511	8.851	4.939	55.8	115	16.7	626	+ 62
17	330	17.51	615.8	84	699.8	24.935	597	5.146	4.701	91.2	107	15.3	704	- 4
18	325	14.23	498.7	84	582.7	23.490	496	6.514	3.950	60.7	74	12.7	570	+ 13
19	325	11.55	475.3	84	559.3	24.988	517	3.917	2.680	68.3	64	11.5	581	- 22
20	327	17.93	608.5	84	692.5	24.682	593	7.548	4.454	58.9	99	14.3	692	0
21	332	16.83	602.8	84	686.8	25.160	474	8.964	5.266	58.7	106	15.4	580	+ 107
22	345	17.32	622.6	84	706.6	23.504	459	8.457	5.428	64.1	105	14.9	564	+ 143
23	350	15.74	561.0	84	645.0	28.182	541	7.891	4.446	56.3	109	16.9	650	- 5
24	352	13.60	549.8	84	633.8	19.413	464	7.987	5.216	65.2	118	18.6	582	+ 52
25	340	9.81	355.7	84	439.7	16.744	414	5.976	3.262	54.5	74	16.8	488	- 48
26	330	10.34	367.8	84	451.8	17.902	448	7.351	4.007	54.5	91	20.1	539	- 87
27	332	5.66	182.6	84	266.6	38.402	363	8.710	4.187	48.1	77	28.8	440	- 173
28	315	Nil	Nil	84	84.0	25.304	349	1.727	1.628	94.1	37	44.1	386	- 302
29	285	Nil	Nil	84	84.0	17.514	264	Nil	Nil	—	Nil	—	264	- 180
30	270	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	—	Nil	—	Nil	—

Table II.

Date	Weight (g.)	Dry food (g.)	Food-N (mg.)	Milk-N (mg.)	Total N intake (mg.)	Weight of urine (g.)	Urine-N (mg.)	Weight of faeces (g.)	Dry weight of faeces (g.)	% of dry matter in faeces	Faecal N (mg.)	Faecal N % of total N intake	Total N output (mg.)	Nitrogen balance (mg.)	C/N ratio
iv. 12	435	16.71	568	84	652	33.03	403	7.13	4.57	64.1	111	17.0	514	+138	0.96
13	445	15.62	524	84	608	27.76	486	7.39	4.03	54.5	93	15.3	579	+29	0.63
14	445	16.17	545	84	629	20.50	521	9.48	4.10	43.3	100	15.8	621	+8	0.67
15	430	12.63	423	84	507	17.87	412	12.37	5.33	43.1	115	22.7	527	-20	0.80
16	430	13.30	444	84	528	18.31	512	8.62	3.98	46.2	100	18.9	612	+84	0.69
17	430	14.50	486	84	570	22.84	424	13.85	5.38	38.8	128	22.4	552	+18	0.65
18	435	15.74	530	84	614	29.22	481	14.64	5.33	36.4	113	18.5	594	+20	0.68
19	437	16.17	544	84	628	23.18	382	14.26	5.75	40.3	130	20.7	512	+116	0.89
20	430	15.39	517	84	601	21.61	323	10.04	5.12	50.0	120	19.9	443	+158	0.84
21	420	—	—	—	—	—	—	—	—	—	—	—	—	—	—
22	410	8.78	293	84	377	17.26	381	10.41	4.13	39.8	95	25.2	476	-99	1.10
23	410	14.07	469	84	553	18.66	296	10.18	3.87	38.0	101	18.2	397	+156	1.20
24	415	13.90	472	84	556	17.37	284	12.73	4.73	37.2	108	19.4	392	+164	1.53
25	417	13.87	466	84	550	19.71	321	12.62	5.78	45.8	145	26.3	466	+84	1.18
26	420	13.07	444	84	528	25.48	390	9.21	4.68	50.9	122	23.1	512	+16	1.09
27	420	10.92	367	84	451	27.94	315	11.31	5.22	46.2	126	27.9	441	+10	1.23
28	415	6.65	233	84	317	16.28	281	12.12	5.71	47.1	134	42.2	415	-98	1.12
29	405	6.25	207	84	291	13.53	275	3.27	2.52	76.8	79	27.1	354	-63	1.02
30	390	5.96	199	84	283	16.74	282	10.13	5.97	59.1	145	51.2	427	-144	1.34
v. 1	395	4.69	158	84	242	19.13	275	7.74	5.06	65.4	119	49.2	394	-152	1.22
2	397	3.12	104	84	188	16.94	358	5.94	3.90	65.7	90	48.4	448	-260	0.99
3	392	2.81	93	84	177	27.37	396	2.95	1.94	65.6	46	25.9	442	-265	0.92
4	375	Nil	Nil	84	84	22.77	262	1.82	1.02	56.0	25	29.9	287	-203	1.33
5	352	Nil	Nil	84	84	28.95	381	Nil	Nil	—	Nil	—	381	-297	0.82

Table III.

Date	Weight (g.)	Dry food (g.)	Food N (mg.)	Milk-N (mg.)	Total N intake (mg.)	Weight of urine (g.)	Urine-N (mg.)	Weight of faeces (g.)	Dry weight of faeces (g.)	% of dry matter in faeces	Faecal N (mg.)	Faecal N % of total N intake	Total N output (mg.)	Nitrogen balance (mg.)	C/N ratio
vi. 15	355	12.39	451	84	535	19.14	286	7.14	4.87	68.2	124	23.2	410	+125	0.92
16	360	12.36	450	84	534	8.51	269	7.26	3.57	49.2	92	17.2	361	+173	0.80
17	360	14.05	510	84	594	22.44	293	8.39	4.49	53.5	118	19.8	411	+183	0.98
18	362	12.30	460	84	544	17.14	276	8.12	5.39	66.3	141	25.9	417	+127	0.85
19	365	14.04	514	84	598	21.58	324	9.10	4.32	47.5	114	19.1	438	+160	0.95
20	370	13.44	488	84	572	23.42	352	9.90	4.49	45.4	113	19.6	465	+107	0.97
21	370	7.39	271	84	355	22.44	262	8.95	4.36	48.7	108	30.4	370	-15	1.16
22	365	9.11	331	84	415	19.35	347	7.83	3.45	44.1	87	20.9	434	-19	0.83
23	360	8.43	303	84	387	20.62	380	6.39	2.80	43.9	67	17.3	447	-60	0.87
24	365	7.83	277	84	361	18.44	339	6.44	3.14	48.8	76	21.1	415	-54	0.84
25	345	7.14	258	84	342	19.22	326	8.41	3.62	43.0	93	27.2	419	-77	0.91
26	345	8.06	286	84	370	24.26	413	7.63	3.58	46.9	92	24.8	505	-135	0.79
27	355	12.56	452	84	536	22.10	370	7.50	3.53	47.1	92	17.2	462	+74	0.84
28	360	14.34	513	84	597	20.38	347	8.71	5.18	59.4	132	22.1	479	+118	0.84
29	365	14.47	523	84	607	24.81	428	8.94	4.49	50.3	117	19.3	545	+62	0.66
30	370	14.99	543	84	627	23.52	397	8.99	4.44	49.4	114	18.2	511	+116	0.78
vii. 1	365	9.63	342	84	426	20.10	340	9.38	4.06	43.3	105	24.7	445	-19	0.75
2	360	6.33	224	84	308	18.60	293	10.29	4.22	41.0	109	35.4	402	-94	0.83
3	350	7.68	277	84	361	22.01	328	9.80	3.64	37.1	96	26.6	424	-63	0.87
4	345	7.39	262	84	346	27.44	327	5.03	2.59	51.4	67	19.4	394	-48	1.09
5	340	3.60	130	84	214	25.06	351	4.18	2.17	51.8	56	26.2	407	-193	0.92
6	330	0.88	31	84	115	20.54	329	3.15	2.30	73.1	58	50.4	387	-273	0.83
7	325	0.61	22	84	106	19.36	292	3.03	1.72	56.6	44	41.5	336	-230	0.92
8	320	Nil	Nil	84	84	20.48	287	2.79	1.90	68.1	47	55.9	334	-250	0.98
9	305	Nil	Nil	84	84	21.54	305	2.50	1.01	40.4	25	29.7	330	-246	0.94
10	300	Nil	Nil	84	84	24.72	340	2.63	1.76	66.9	44	52.4	384	-300	0.90
11	295	Nil	Nil	84	84	21.98	314	2.01	1.46	72.6	36	42.8	350	-266	0.93
12	285	Nil	Nil	84	84	14.52	258	2.16	1.62	75.0	39	46.4	297	-213	0.85
13	285	7.49	270	84	354	16.44	277	4.03	2.28	56.6	52	14.7	329	+25	0.94
14	290	10.02	362	84	446	20.79	320	5.83	2.71	46.5	63	14.1	383	+63	1.08
15	305	12.53	452	84	536	22.74	307	8.91	4.44	49.8	113	21.1	420	+116	0.99
16	310	12.33	438	84	522	21.73	300	8.83	4.68	53.0	116	22.2	416	+106	1.01
17	310	11.78	424	84	508	20.20	272	9.01	4.42	49.1	105	20.7	377	+131	1.06
18	315	11.57	422	84	506	20.14	300	12.13	4.74	39.0	112	22.1	412	+94	0.84
19	317	12.36	447	84	531	20.42	289	12.70	5.58	43.9	136	25.6	425	+106	0.94
20	320	11.82	424	84	508	23.32	275	10.52	5.52	52.5	129	25.4	404	+104	1.17
21	322	12.86	456	84	540	16.26	286	13.41	6.21	46.3	147	27.2	383	+157	1.00
22	325	12.16	443	84	527	17.61	287	9.32	4.38	46.9	104	19.7	391	+136	0.93
23	327	11.67	423	84	507	20.40	331	9.85	3.96	40.2	87	17.2	418	+89	0.91
24	327	11.99	431	84	515	24.72	371	10.39	5.09	49.0	110	21.3	481	+34	0.89

dry matter evacuated in the faeces (4.6 g.) was roughly a fourth of that ingested in the food (15.7 g. of the food mixture and about 5 g. of the dry milk), whilst about a sixth of the nitrogen ingested was excreted in this way. In the second period the food, mainly the basal mixture, was restricted so that the animal lost in weight, the quantity administered daily being controlled by the weight of the animal. The same dose of decitrated lemon juice was given in this period as in the preceding one, thus keeping the animal fully protected from scurvy.

Table IV.

Period	...	...	...	I	II	III	IV
Dry food (g.)	...	...	...	15.7	9.3	16.6	14.1
Nitrogen intake (mg.)	...	...	...	614	357	678	449
Urine (g.)	...	...	...	24.8	20.7	25.2	22.6
Urinary nitrogen (mg.)	...	...	...	449	352	465	414
Dry faeces (g.)	...	...	...	4.6	2.9	4.8	4.3
Faecal nitrogen (mg.)	...	...	...	105	66	112	73
Faecal % N of total N intake	...	...	...	18.0	19.1	16.8	15.7
Nitrogen balance (mg.)	...	...	...	+60	-61	+101	-38

Period I. 28 days; basal diet + 5 cc. decitrated lemon juice.

" II. 8 days; restricted quantities of basal diet + 5 cc. decitrated lemon juice.

" III. 10 days; basal diet + 5 cc. decitrated lemon juice.

" IV. 12 days; basal diet + 5 cc. water.

In examining the figures of this period, it is seen, as one would expect, that there was a diminution in the amount of dry matter and nitrogen ingestion. The excretion of dry matter in the faeces and of nitrogen in the faeces and in the urine was accordingly diminished. The ratios, however, of the excreted dry matter or of nitrogen to those consumed did not alter to any great extent. The nitrogen balance of this period was, of course, negative. The eight days of restricted feeding were then followed by a recuperative period of 10 days, during which time the guinea-pig was fed as in period I. The figures obtained during this time resemble, in general, those of the first period. In the final period the decitrated lemon juice was discontinued and replaced by an equal volume of water. The animal was consequently receiving a scorbutic diet. During the first 9 days the guinea-pig progressed well, but at this stage it developed pneumonia, from which it died 4 days later. During the last 2 days it ceased consuming the food mixture. At the *post mortem* examination definite ridging of the costochondral junctions was found, but no other macroscopic scorbutic lesions were detected. It will be seen that during the 8 days of the scorbutic period, the intake and output of the food and nitrogen were proceeding more or less as in periods I and III, and the relationship of the output of the dry matter and of the nitrogen in the faeces to their intake is not very different from that in the other periods. The nitrogen balance was again roughly parallel to the growth of the animal. Only during the last 3 or 4 days was there a marked negative balance which was due to the very rapid premortal decline.

Guinea-pig No. 2 (Tables II and V). After a preliminary period of 6 days on the basal diet and 5 cc. of decitrated lemon juice, the antiscorbutic was

replaced by an equal volume of water. During the first 10 days of the scorbutic period the animal consumed its food well but after that time the intake gradually diminished and after a further 6 days there was a cessation in the consumption of the food mixture, the animal dying 2 days later. At the *post mortem* examination pneumonia, slightly swollen knee joints, haemorrhages in the gastrocnemius and femoral muscles of both legs and slightly enlarged costochondral junctions were established. In examining the intake and output figures during the first 10 days of the scorbutic period, when the consumption of food was normal, no striking differences are found between them and those of the preliminary period. The output of dry matter and of nitrogen in relation to their intake is slightly higher but this is of an order to which no significance can be attached. As in the preceding animal, there was a very marked negative nitrogen balance during the decline in the premortal phase.

Table V.

Period	...	...	I	II
Dry food (g.)	...	...	14.8	13.5
Nitrogen intake (mg.)	...	...	583	519
Urine (g.)	...	...	23.4	22.3
Urinary nitrogen (mg.)	...	...	459	345
Dry faeces (g.)	...	...	4.5	4.9
Faecal nitrogen (mg.)	...	...	108	118
Faecal N % of total N intake	...	...	18.7	22.1
Nitrogen balance (mg.)	...	...	+16	+56

Period I. 6 days; basal diet + 5 cc. decitrated lemon juice.

„ II. 10 days; basal diet + 5 cc. water.

Table VI.

Period	...	...	I	II	III	IV
Dry food (g.)	...	...	13.0	9.9	0.6	11.5
Nitrogen intake (mg.)	...	...	562	440	104	499
Urine (g.)	...	...	17.9	21.8	21.0	20.4
Urinary nitrogen (mg.)	...	...	291	349	309	298
Dry faeces (g.)	...	...	4.5	3.8	1.7	4.5
Faecal nitrogen (mg.)	...	...	118	98	44	106
Faecal N % of total N intake	...	...	21.0	22.9	43.2	20.9
Nitrogen balance (mg.)	...	...	+153	-7	-249	+95

Period I. 5 days; basal diet + 5 cc. decitrated lemon juice.

„ II. 15 days; basal diet + 5 cc. water.

„ III. 8 days; 6 days on basal diet + 5 cc. water and 2 days on basal diet + 5 cc. decitrated lemon juice.

„ IV. 12 days; basal diet + 5 cc. decitrated lemon juice.

Guinea-pig No. 3 (Tables III and VI). This animal had a preliminary period of 5 days, followed by a scorbutic period of 21 days, in which the decitrated lemon juice was replaced by water, and eventually by a curative period in which the decitrated lemon juice was resumed. In the very early part of the scorbutic period there was, for some unknown reason, a diminished consumption in the food mixture for a few days, but the animal soon resumed its normal consumption until the onset of the early symptoms of scurvy when the intake began diminishing as before until no consumption of the mixture was recorded. The diminution in the food consumption during the very early

stages of the scorbutic period has fortuitously proved of use for comparison with a short period when the consumption was of the same order between 10th and 15th day and when the animal was undoubtedly scorbutic. By examining the nitrogen balance of these two periods one finds a great similarity, namely, a small negative balance caused by an insufficient intake of nitrogen. There is no indication that the presence of incipient scurvy made any difference in this respect. Again, when one examines the average figures for the first 15 days of the scorbutic period (Table VI, period II) one finds that in spite of the somewhat diminished intake of the food and of nitrogen, the ratios of the output of total solids and of nitrogen in the faeces to their intake is not appreciably different from those of the first and last periods. Between the 15th and 21st day of the scorbutic period and during the first 2 days of the last period, owing to the extremely small consumption of the basal mixture, the above ratios are abnormally high and the balance is markedly negative. The figures of the last period following the recovery from scurvy are very similar to those of the preliminary period.

CONCLUSIONS.

It will be seen from the above results that, in spite of all care, untoward complications, such as the intervention of pneumonia, could not be prevented. Disconcerting as this may be, it does not influence the results in such a way as to prevent them from affording the information which they were intended to yield, namely, whether there is any association between the onset of scurvy and a change in the absorption and retention of nitrogen. It was assumed that if this were the case it would manifest itself at an early stage of the animal's existence on a scorbutic diet, since it has been shown that macroscopic scorbutic lesions are in evidence approximately 10 days after the guinea-pig has been placed on the scorbutic diet, whilst microscopic changes may be observed even earlier [Zilva and Wells, 1919; Höjer, 1924]; in fact, one of us has had the opportunity of observing small subcutaneous haemorrhages in some cases as early as the 6th day. Daily observations on the intake and output of nitrogen would, therefore, bring to light even an abrupt change of short duration before this could be vitiated by the superimposition of the changes due to starvation. In analysing the above results one does not find even a suggestion that this is the case. In turning our attention to the figures dealing with the retention of nitrogen we see that in all the experiments the nitrogen balance is generally controlled by the amount of food consumed and in consequence by the growth of the animal in some instances, and is independent of the scorbutic condition of the animal. In the premortal phase or when the scurvy is acute, the balance is markedly negative, not because of the scurvy but as a consequence of starvation caused by the disease, either alone or complicated with pneumonia. Were the syndrome of scurvy a sequel to a disturbed nitrogen retention caused by the vitamin deficiency it would become evident not much later than the 10th day. The accidental similarity of the food intake of guinea-

pig No. 3 between 20th and 27th June and between 30th June and 5th July shows further that this is not the case. Similarly, the results fail to show a diminished assimilation of nitrogen. This is particularly evident from the tables of averages which show that there is no striking difference in the relation between the faecal nitrogen and the total nitrogen intake of the scorbutic and normal periods. A difference is observed only when the intake of the food mixture is almost nil, as occurs during the few days preceding death or during period III of guinea-pig No. 3, which is evidently the effect and not the cause of scurvy. The C/N ratios in the urine of guinea-pigs 2 and 3 were not concordant; in the first animal there was a definite increase in the ratio soon after the animal was placed on the scorbutic diet. This increase persisted until the death of the animal. No such deviation was observed in the case of guinea-pig No. 3.

As far as we are aware the only investigations which have been carried out on the nitrogen balance of scorbutic guinea-pigs in relation to normal animals have been recorded by Baumann and Howard [1917] and by Jarussowa [1928]. The former investigators have noted a high negative balance during the scorbutic period but, as their animals consumed an insufficiency of nitrogen and of calories during this period, a negative balance was to be expected. Jarussowa tried to overcome these difficulties. Briefly, her procedure consisted in forcibly feeding the guinea-pigs, which were kept in a metabolism cage, with powdered oats in the form of pills and boiled cabbage juice. Figures for a series of periods, but not daily figures, are given by her. It transpires from these data that the animal maintained its initial weight of 396 g. more or less steadily for 24 days, after which time it began losing weight rapidly. During these 1-24 days all the periods show a small positive balance. The following two periods are marked by an increasing and significant loss (18.2 %) in weight with a corresponding negative balance which lasted to the end of the experiment. The total daily nitrogen intake except for the last 2 days lay in the neighbourhood of 500 mg., the daily output in the urine was a little less than 400 mg. and showed little variation, whilst the nitrogen output in the faeces was of the order 100 mg. per day for the first 18 days with about a 20 % increase during the remainder of the experiment. This picture is not unlike the one we have obtained. We do not, however, agree with Jarussowa that "...während der Skorbutentwicklung geht die Stickstoffbilanz von einer positiven Grösse zu einer negativen über..." Scurvy in the young guinea-pig does not develop on the 24th day, when the change in the nitrogen balance occurred in these experiments. At this time the disease is of such severity as to disturb seriously all the functions of the animal organism. For the same reason we do not think that the higher nitrogen content of the faeces during the last 12 days of the experiment, even if it be a regular feature, which our experiments make us doubt, is caused directly by scurvy. Jarussowa also observed that the C/N ratio of the urine doubled between the 18th and 29th day and then became normal again until the end of the experiment. This

observation taken in conjunction with the discordant results we have obtained in this respect calls for further experiments in this direction. It seems, however, unlikely, from both investigations that the development of scurvy is associated with an increased C/N ratio in the urine.

SUMMARY.

There are no indications of a disturbed absorption or retention of nitrogen during the early stages of the development of scurvy in growing guinea-pigs. The nitrogen balance becomes negative only when the intake of food is diminished as a consequence of the disease. Although the results obtained were not concordant, it is unlikely that the C/N ratio of the urine of young guinea-pigs is disturbed by the onset of scurvy.

REFERENCES.

- Baumann and Howard (1917). *Amer. J. Med. Science*, **53**, 659.
Gross and Connell (1923). *J. Physiol.* **57**, Proc. ix.
Höjer (1924). *Acta Paediatrica*, **8**.
Jarussowa (1928). *Biochem. Z.* **198**, 128.
Zilva and Wells (1919). *Proc. Roy. Soc. Lond. B*, **90**, 505.