

Feeding Vitaminized Peanut Butter to Rats to Confirm Chemical Assays of Vitamin A.

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CHEMICAL assays for Vitamin A in products of animal origin, have been utilized by many workers since the colour tests of Carr-Price (1926) were standardised and the extraction and evaluation of the vitamin was advocated by Moore (1930). Since then the standardised chemical method has been found to be of practical value giving valid results, which may not actually be precise, but time-saving, simple and cheap, compared with the laborious, expensive, but more accurate biological methods.

Evidence has been presented by various workers, Rosenheim and Webster (1927), Wokes and Willmott (1927), Ahmad and Drummond (1930), Moore (1931), to show the practical value of the colour reaction as a means to assay Vitamin A in liver oils, etc.

The purpose of the present work was to obtain confirmation for results obtained on vitaminized peanut butter by the application of the chemical method. Repeated assays, on samples of this butter to which vitamin A had been added in the form of fish liver oils in the processing, gave an average value of 69 International (I.U.) per gram peanut butter. In employing the biological tests, the doses given were calculated from this value, which was obtained by chemical means. Thus small doses of 30 mgm. then represent 2.1 I.U. of vitamin A per dose.

EXPERIMENTAL.

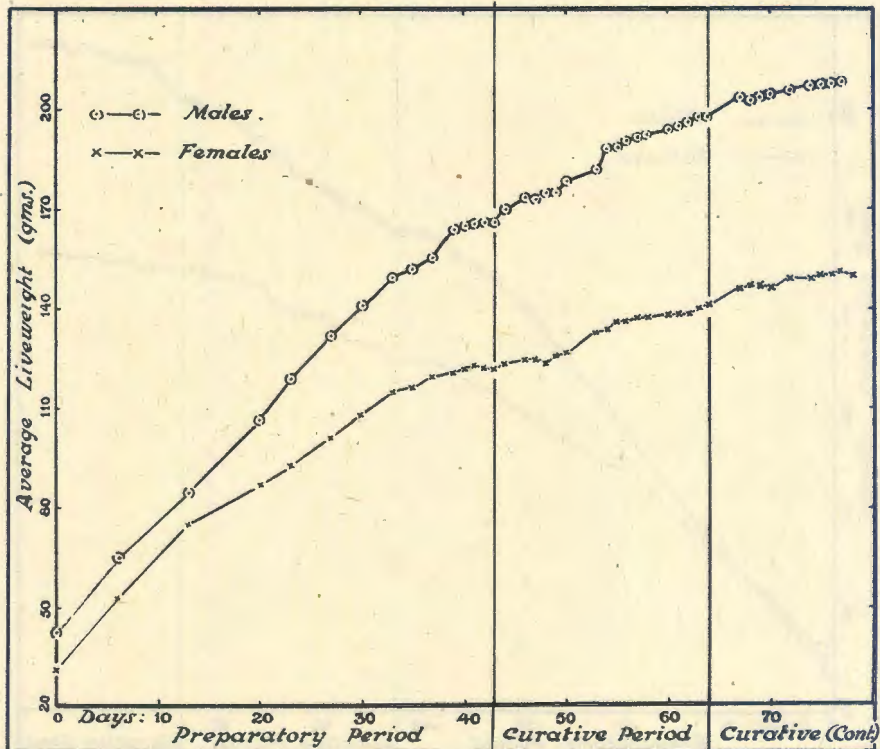
Series No. 1.

Young rats (males and females) at weaning stage and three weeks old, were used. The rats were of nearly the same age and had low reserves of vitamin A in their livers. This was established by slaughtering a few rats and an average value of about 10 I.U. of vitamin A for the whole liver was found.

Precautions were taken to ensure this low reserve by feeding the mother rats the deficient diet after the second week of lactation; the young sucklings could thus not enhance their reserves by nibbling the feed or feeding thereon. It is very essential that the young rats should not have too high a reserve of vitamin A in the livers, as pointed out by various workers (e.g., Davies and Moore, 1937), since the depletion period would thereby be unnecessarily prolonged, and may be extended from weeks to months. That the young rats do build up a substantial reserve in their livers when feeding on a diet rich in vitamin A, was indicated by these authors (1937).

doses were weighed out on glass slides in flat bottom glass basins (Petrie Basins), so that the rat could not take the slide in its mouth. In this way, by close observation, one could make sure that the animal had consumed all. By moistening the butter with a drop or two of olive oil, the rats gave no trouble at all and readily devoured the small quantity of butter given them. Weighings were carried out, at first weekly, then bi-weekly, and in the last stages of the preparatory period, when the weights became constant, daily. In the Curative Period the weighings were carried out every second day or daily.

GROUP A (Series No. 1).



Series No. 2.

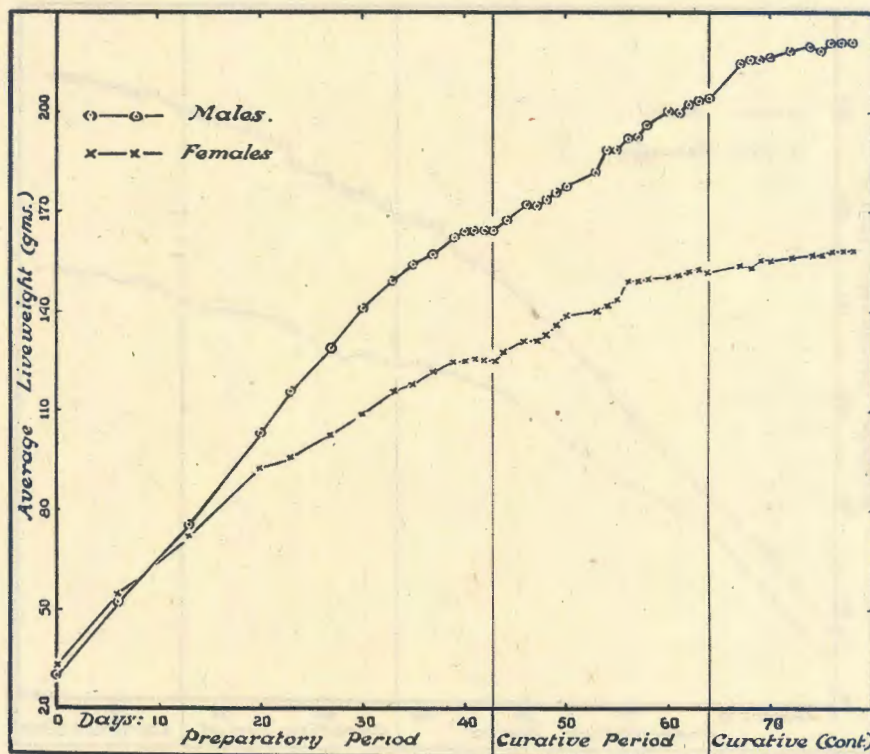
In a second series of tests where rats were given vitamin A, improvements were made on Series No. 1.

Here equal numbers of males and females were used in each group, which was increased by one animal. In the three groups there were thus six animals each. Group D was the control (where no vitamin A was given). Group E was given vitaminized peanut butter at the rate of 60 mgm. (=4.2 I.U. Vit. A) per rat daily. Group F was given 120 mgm. (=8.4 I.U. Vit. A) per rat daily.

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It was obvious from the preliminary tests that waiting overlong for the rats to become steady in weight in the preparatory period, before initiating the curative period, resulted in a slow response of some rats to curative treatment. There was a lag in growth response and the rats concerned could not compete with their group mates. As a result such a group, where this setback occurred, could not compete with another group where no such setback was found. The females were most liable to such behaviour. In the 2nd series, the rats were carefully observed and when about 60 per cent. of animals became steady in weight for about three days the curative period was begun.

GROUP B (Series No. 1).



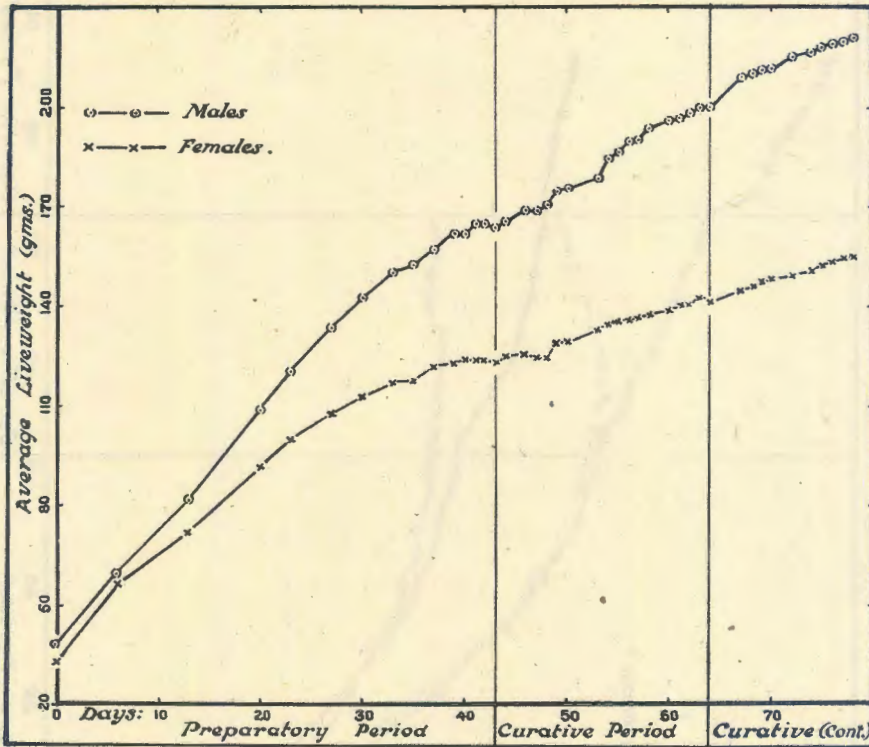
From the growth curves (for 5 weeks dosing) in the previous series, it was evident that doses of 2.1 I.U. vitamin A per rat daily (for fish oil as well as peanut butter) were too small to yield normal growth curves. Hence doses of double strength, viz., 4.2 I.U. and also 8.4 I.U. were taken.

Discussion.

Positive growth results were obtained when vitaminized peanut butter had been fed to rats previously depleted of their vitamin A reserves. The depletion on preparatory periods lasted 43 days in the case of Series No. 1, and 52 days in Series No. 2. The curative period was 21 days and there was response in every case where the peanut butter had been dosed. By

prolonging the curative periods for 35 days without a break, it could be established whether normal growth was taking place. In Series No. 1 the rats of Groups A and B grew on an average sub-normally, as compared with Group C notwithstanding the fact that a few rats of this group lagged behind in body weight returns in the initial stages of curing. Group B appeared to be best favoured in this respect and there was a quick growth response to vitamin A. In Series No. 2, positive growth responses were found in both Groups E and F, where vitamin A had been given, but Group D (controls)

GROUP C (Series No. 1).



showed in most cases loss in body weight or a tendency to cease growing or to grow irregularly with small gains for the period. In these young rats of less than 3 months of age, typical symptoms of vitamin A deficiency were observed which are here described.

Group D.

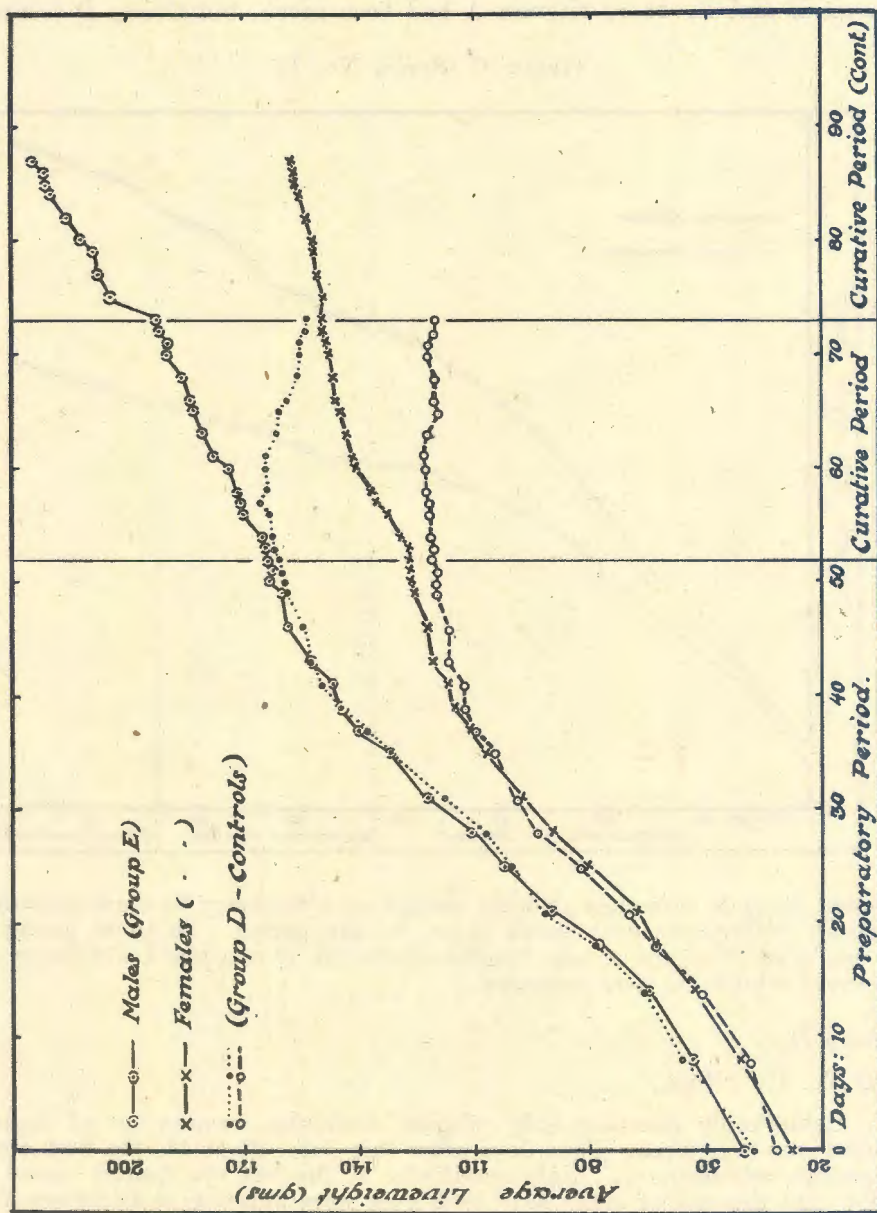
Rat No. 1. (Male.)

Remarkably constant body weight, since the termination of depletion period was registered. Four days after this date (23.10.41) the first obvious symptom was seen, viz., light-sensitivity of the left eye (partly closed eyelids). At the end of eight days both eyes were affected; a discharge of the right eye was apparent; deterioration of the eye gland was taking place.

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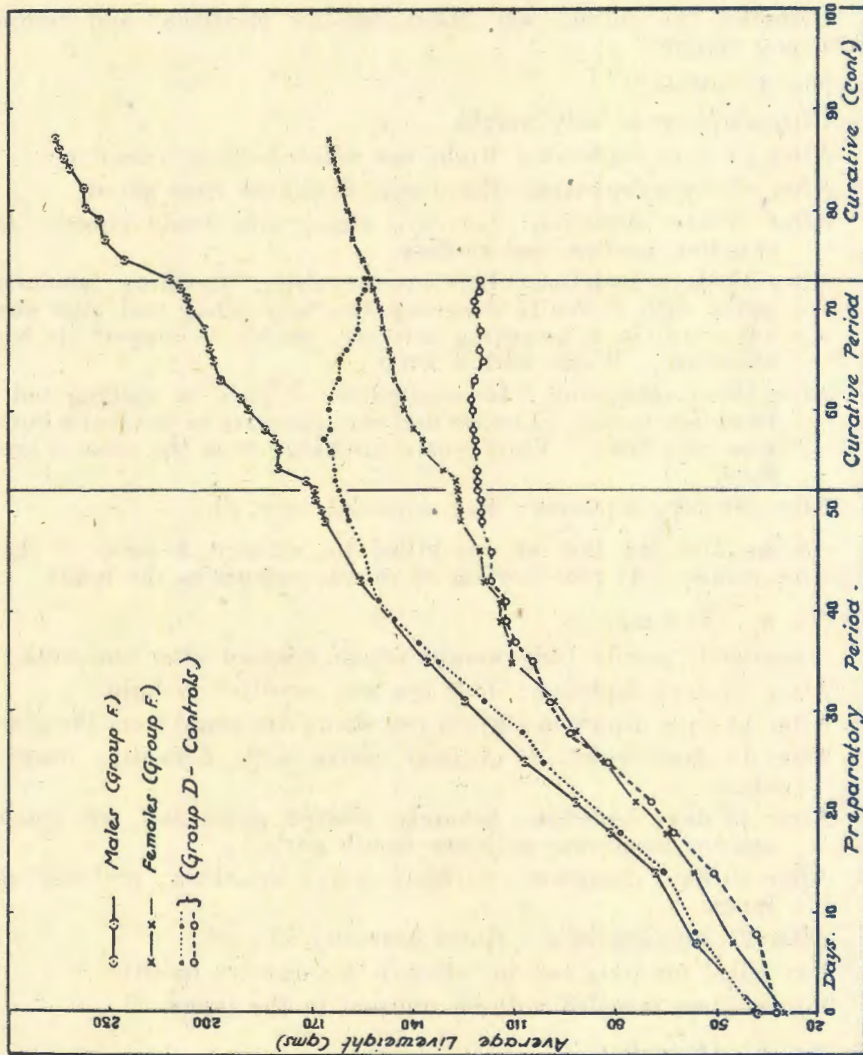
The next day, the rat lost its appetite which did not return before vitamin A curative dosing was practised at a later stage. On the 13th day the rat was listless, and obviously ill. The eyes were dull and keratinization of the eyelids was observed. On the 15th day, difficulty to walk normally, due partly to inactivity and partial lameness (paresis) was seen. On examination of the mucous membrane of the lower jaw, tainted gums of incisors

GROUP E (Series No. 2).



were observed. A catarrhal discharge of the nose, sticky and brown in colour, appeared on the 19th day. As the rat was very ill and would have died since it was wasting away quickly, curative treatment by dosing diluted fish liver oil, was practised. The rat revived, appetite was restored, body weight increased remarkably, and the general health returned. After a short period of four days, the rat increased its body weight by 10 grams due largely to an enhanced appetite.

GROUP F (Series No. 2).



Rat No. 2. (Male.)

Body weight showed an ultimate drop.

After 10 days depletion: Right eye affected.

After 13 days depletion: Light-sensitivity.

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After 15 days depletion: Animal crouched and became listless.

After 17 days depletion: The animal showed paresis of the hind legs.

After 18 days depletion: Appetite impaired. There was a catarrhal discharge of left nasal chamber. Gums were bloody on lower jaw.

After 19 days depletion: Increased nasal discharge.

After 20 days depletion: Rat apparently deaf.

Hereafter the animal was given curative treatment and restored to health and vigour.

Rat No. 3. (Male.)

Ultimate drop in body weight.

After 4 days depletion: Right eye affected—light sensitive.

After 6 days depletion: Right eye, discharge from gland.

After 9 days depletion: Left eye almost completely closed. Animal twitches, nervous and restless.

After 17 days depletion: Left eye purulent; breathing laboured; rat walks with difficulty dragging the body along and goes down on haunches, in a sprawling attitude, unable to support its body on hind legs. Walks with a limp.

After 19 days depletion: Inco-ordination of limbs in walking and sways from side to side. Listless and very sensitive to the touch but otherwise very tame. There was a discharge from the nose of brownish fluid.

After 20 days depletion: Rat appeared very ill.

On the 21st day the rat was killed for vitamin A assay of the liver (negative result). At post-mortem there was oedema of the lungs.

Rat No. 4. (Female.)

Remarkably steady body weight which dropped after one week.

After 8 days depletion: Left eye was sensitive to light.

After 14 days depletion: Right eye shows discharge from the gland.

After 16 days depletion: Animal walks with difficulty, drags body along.

After 18 days depletion: Severely affected gums and red patches on mucous membrane of lower mouth parts.

After 19 days depletion: Sniffles; husky breathing, probably affected lungs.

After 21 days depletion: Gums generally affected.

Rat killed for liver test for vitamin A (negative result).

Post-mortem revealed nothing unusual in the lungs.

Rat No. 5. (Female.)

Rather irregular, but small gain in body weight.

After 13 days depletion: Right eye light sensitive.

After 16 days depletion: Twitching of muscles. Walks with difficulty.

After 18 days depletion: Appetite had been fairly good all along but now apparently lost. Discharge, though scant, of the nose.

After the 21st day the animal was given vitamin A (oil) orally. A considerable gain in body weight was observed, 11 grams in 4 days, due to enhanced and restored appetite. The rat was quick in response to dosing and showed brightness of the eyes in comparison with previous dullness.

Rat No. 6. (Female.)

Showed about 7 grams increase in body weight for the three weeks. No obvious symptoms, except for a slight discharge of the right eye on the 20th day.

The rat was hereafter dosed with vitamin A and restored to vigorous growth. In 4 days of vitamin A administration the animal gained 11 grams in body weight.

LIVER ASSAYS.

In order to ascertain what reserves (if any) of vitamin A were stored in the livers during the curative periods, at the various stages, liver assays were carried out as follows:—

In both Series No. 1 and No. 2:

- (a) 2 rats (male and female) were slaughtered at weaning (three weeks after birth).
- (b) 2 rats (male and female) were slaughtered at the end of preparatory periods.
- (c) 2 rats in each group (A-C and E-F) at the end of 21 days of curative period.
- (d) the residual rats of each group at the end of 5th week of vitamin A supplementation (see Tables 3 and 6 in the appendix).

Small reserves of vitamin A were found at the initial stages. Storage had taken place during the gestation period and lactation period of the mothers. Due to controlled feeding of vitamin A to the mother, the reserve of vitamin A in the young sucklings was kept low (about 10 I.U.).

At the end of preparatory periods no vitamin A could be found on testing the liver reserves proving that in all the rats the livers had probably been thoroughly depleted in the short time of 43-52 days.

At the end of the curative period of 21 days no vitamin A had been stored in the liver in Groups A, B and C in Series No. 1. Hence 2.4 I.U. of vitamin A per rat daily were, therefore, enough to prevent symptoms and grant a certain level of growth but allowed no reserves to be built up in this time. In Series No. 2, however, a small reserve was stored in the livers of the rats in Group F at the end of 21 days but none in the rats of Group E. In other words, where 4.2 I.U. vitamin A per rat daily was insufficient to allow a reserve to be stored in the livers after 21 days, 8.4 I.U. vitamin A per rat daily allowed a small quantity in reserve. Again, after the expiration of 5 weeks of the curative period, the rats slaughtered (Groups E and F) showed no storage in Group E and a small storage in Group F. The female rat of the last-named group had stored as much as 3.6 I.U. vitamin A in the whole liver. The other members of this group had only stored a small reserve of about 0.6 I.U. due largely to the requirements being larger and thus the demand was bigger than for the female, especially where the male

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registered a rate of growth of 85 gm. in 5 weeks. It is to be noted that the rats of the control group, when cured after the expiration of 21 days during which time typical symptoms were observed, very quickly responded to treatment and gained rapidly in weight thereafter.

In conclusion, the daily doses of 4.2 I.U. and 8.4 I.U. vitamin A calculated from the value 1 gm. = 69 I.U.s. based on chemical analyses, were found by biological tests to be adequate to give positive growth responses, as can be gathered from the graphs. Chemical analyses are thus valuable indications of the vitamin A value of foods.

SUMMARY.

Positive results were obtained when vitaminized peanut butter was fed in small doses of 4.2 and 8.4 I.U.'s. of vitamin A per rat daily. Body weight increases served as criteria. For the same peanut butter, on chemical tests an average value of 69 I.U.'s. of vitamin A per gram was found at the initial stages of the biological tests.

The doses were calculated on the chemical findings and on this basis doses of 2.1 I.U.'s were too small, whilst doses of 4.2 I.U. were adequate; and doses of 8.4 also allowed a small storage in the liver within 21 days of feeding. The above findings support the views of Goss and Guilbert (1939) who advocated a minimum level of vitamin daily of 18-22 I.U. per Kgm. weight in rats, which, calculated on the average weight of the rats in this experiment is equivalent to 2.5-3.9 I.U. vitamin A per dose per rat daily.

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TABLE I.
Body Weight of Rats (Gm.).
Preparatory Period.—Series No. 1 (Duration 43 Days).

Rat No.	Sex.	15/8/41.	20/8/41.	27/8/41.	3/9/41.	6/9/41.	10/9/41.	13/9/41.	16/9/41.	18/9/41.	20/9/41.	22/9/41.	23/9/41.	24/9/41.	25/9/41.	26/9/41.
1	Male.....	64	83	99	119	133	145	149	157	160	164	168	170	170	170	170
2	Male.....	30	53	84	110	124	136	148	159	165	171	176	177	178	179	180
3	Male.....	32	58	73	88	99	116	126	135	139	142	147	147	148	148	148
4	Female.....	35	56	79	88	94	102	111	118	119	123	123	124	126	125	125
5	Female.....	28	51	71	86	93	100	105	112	113	117	119	120	120	119	120
Average.	Female.....	42	65	85	106	119	132	141	150	155	159	164	165	166	166	166
	Female.....	31	53	75	87	93	101	108	115	116	120	121	122	123	122	122
6	Male.....	29	59	85	108	118	132	142	145	149	151	155	155	156	156	155
7	Male.....	29	50	69	97	150	118	129	139	144	147	152	154	156	156	156
8	Male.....	32	48	70	104	124	137	152	164	169	173	178	182	181	181	182
9	Female.....	34	60	80	100	101	104	110	118	119	123	126	126	127	125	125
10	Female.....	32	50	64	86	94	102	109	114	117	121	124	124	127	125	126
Average.	Male.....	30	52	75	103	116	129	141	149	154	157	162	164	164	164	164
	Female.....	33	55	72	93	97	103	109	116	118	122	125	125	126	125	125
11	Male.....	51	71	95	123	137	153	161	168	169	172	176	177	179	179	178
12	Female.....	34	56	71	98	110	124	134	140	146	151	156	155	158	158	157
13	Male.....	30	54	81	107	116	126	134	144	145	151	153	153	157	157	157
14	Female.....	34	56	65	90	98	108	114	120	122	125	127	128	128	127	126
15	Female.....	33	60	77	93	101	108	112	115	115	119	120	120	121	120	120
Average.	Male.....	38	60	82	109	121	134	143	151	153	158	162	162	165	165	164
	Female.....	33	58	72	92	100	108	113	117	118	122	123	124	124	124	123

TABLE 2.
Body Weight of Rats (Gm.).
Curative Period.—Series No. 1 (Duration 21 Days).

Rat No.	Sex.	26/9/41.	27/9/41.	29/9/41.	30/9/41.	1/10/41.	2/10/41.	3/10/41.	6/10/41.	7/10/41.	8/10/41.	9/10/41.	10/10/41.	11/10/41.	13/10/41.	14/10/41.	15/10/41.	16/10/41.	17/10/41.	Gains.*	Remarks.		
Group A. (2 I.U. Vit. A. per rat daily).	1	Male.....	170	174	179	181	181	183	185	190	191	192	194	193	194	195	196	196	196	+26	Curative period continued on to 5 weeks.		
	2	Male.....	180	183	186	185	187	187	192	194	198	200	202	203	204	206	207	208	210	213	+30	Curative period continued on to 5 weeks.	
	3	Male.....	148	152	156	155	158	161	163	169	177	177	179	180	182	184	185	187	189	192	+41	Killed for Liver Vit. A. Assays.	
	4	Female....	125	125	129	129	124 [†]	127	130	134	136	137	139	141	140	140	141	140	144	144	146	+18	Killed for Liver Vit. A. Assays.
	5	Female....	120	124	122 [†]	121	123	124	126	128	130	130	133	133	134	136	135	137	137	137	137	+16	Curative period continued on to 5 weeks.
Group B. (2 I.U. Vit. A. per rat daily).	Average.	Male.....	166	170	174	173	175	175	182	189	189	191	192	193	194	195	196	198	198	+32	—		
	Female....	122	124	125	125	124	126	128	133	134	136	136	137	137	138	138	138	140	141	+17	—		
	Male.....	155	158	157	152 [†]	155	156	158	161	168	168	170	172	176	180	180	181	183	186	+28	Killed for Liver Vit. A. Assays.		
	Male.....	156	160	166	168	171	173	175	181	187	188	191	192	195	198	198	201	202	201	+45	Curative period continued on to 5 weeks.		
	Male.....	182	184	192	192	195	198	200	204	212	212	215	216	217	223	223	225	226	227	+44	Curative period continued on to 5 weeks.		
Group C. (4 I.U. Vit. A. per rat daily).	Female....	125	129	131	130	132	134	137	138	139	145	149	148	150	150	150	153	150	150	+27	Killed for Liver Vit. A. Assays.		
	Female....	126	128	131	132	134	139	140	143	145	144	149	149	150	151	152	152	153	152	+26	Curative period continued on to 5 weeks.		
	Male.....	164	167	172	171	174	176	178	182	189	189	192	193	196	200	200	203	204	205	+39	—		
	Female....	125	128	131	131	133	136	139	140	142	144	149	149	150	150	151	152	153	152	+26	—		
	Male.....	178	178	181	183	185	190	191	197	201	202	206	207	210	212	211	214	217	215	+37	—		
Male.....	157	161	163	160 [†]	160	163	164	167	173	173	177	180	180	184	184	185	187	189	190	+32	—		
Male.....	157	158	164	165	169	171	172	174	180	181	184	184	187	189	193	193	194	196	196	+38	—		
Female....	126	129	133	130 [†]	130	135	133 [†]	136	135 [†]	135	136	137	138	138	138	139	140	143	143	+16	—		
Female....	120	123	120 [†]	120	121	126	127	130	136	136	136	137	137	139	140	140	141	143	142	+22	—		
Average.	Male.....	164	166	169	169	171	175	176	179	185	187	190	191	194	196	196	198	200	200	+36	—		
Female....	123	126	126	125	130	130	133	133	135	136	136	137	138	138	139	140	141	143	142	+19	—		

* Gains calculated on the average weights for last 3 days.

† Rats suffered temporary setback.

TABLE 2 (a).
Body Weight of Rats (Gm.).
Continuation of Curative Period (to 35th day)—Series No. 1.

Rat No.	Sex.	20/10/41.	21/10/41.	22/10/41.	23/10/41.	25/10/41.	27/10/41.	28/10/41.	29/10/41.	30/10/41.	31/10/41.	Remarks.
1	Male.....	196	197	197	197	200	201	200	201	202	204	Total gain (35 days) = + 34 gm.
2	Male.....	212	209	211	212	212	213	214	214	215	214	Total gain (35 days) = + 34 gm. Rat killed for Liver test.
4	Female....	146	147	147	146	149	149	150	150	151	150	Total gain (35 days) = + 25 gm. Rat killed for Liver test.
7	Male.....	203	203	202	204	205	206	204	205	205	204	Total gain (35 days) = + 48 gm. Rat killed for Liver test.
8	Male.....	227	228	229	230	233	235	234	236	236	237	Total gain (35 days) = + 55 gm.
10	Female....	154	153	155	155	156	156	157	160	160	160	Total gain (35 days) = + 34 gm. Rat killed for Liver test.
11	Male.....	218	218	219	220	222	222	224	226	226	228	Total gain (35 days) = + 50 gm. Rat killed for Liver test.
13	Male.....	200	201	203	203	208	209	212	212	213	216	Total gain (35 days) = + 59 gm. Rat killed for Liver test.
14	Female....	145	146	148	149	149	151	153	154	155	155	Total gain (35 days) = + 29 gm.

TABLE 3.

Liver Assays for Vitamin A Reserves. Rats in Series No. 1.

Rat No.	Sex.	PREPARATORY PERIOD.						CURATIVE PERIOD.									
		Initial Stage.			End Stage.			After 21 days.			After 35 days.						
		Weight.	Liver.	Vit. A.	Storage I.U.	Weight.	Liver.	Vit. A.	Storage I.U.	Weight.	Liver.	Vit. A.	Storage I.U.	Weight.	Liver.	Vit. A.	Storage I.U.
		Gms.	Gms.	I.U.	I.U.	Gms.	Gms.	I.U.	I.U.	Gms.	Gms.	I.U.	I.U.	Gms.	Gms.	I.U.	I.U.
—	Male.....	40	2.1	12.0	12.0	—	—	—	—	—	—	—	—	—	—	—	—
—	Female.....	27	1.4	13.4	13.4	—	—	—	—	—	—	—	—	—	—	—	—
16	Male.....	—	—	—	—	159	8.1	0	0	—	—	—	—	—	—	—	—
17	Female.....	—	—	—	—	125	6.7	0	0	—	—	—	—	—	—	—	—
3	Male.....	—	—	—	—	—	—	—	—	189	9.8	0	0	—	—	—	—
5	Female.....	—	—	—	—	—	—	—	—	136	6.3	0	0	—	—	—	—
6	Male.....	—	—	—	—	—	—	—	—	183	8.6	0	0	—	—	—	—
9	Female.....	—	—	—	—	—	—	—	—	152	7.5	0	0	—	—	—	—
12	Male.....	—	—	—	—	—	—	—	—	189	10.3	0	0	—	—	—	—
15	Female.....	—	—	—	—	—	—	—	—	142	5.8	0	0	—	—	—	—
2	Male.....	—	—	—	—	—	—	—	—	—	—	—	—	214	8.0	0	0
4	Female.....	—	—	—	—	—	—	—	—	—	—	—	—	150	6.5	0	0
7	Male.....	—	—	—	—	—	—	—	—	—	—	—	—	205	9.7	0	0
10	Female.....	—	—	—	—	—	—	—	—	—	—	—	—	159	7.0	0	0
11	Male.....	—	—	—	—	—	—	—	—	—	—	—	—	227	8.0	0	0
14	Female.....	—	—	—	—	—	—	—	—	—	—	—	—	155	6.0	0	0

FEEDING VITAMINIZED PEANUT BUTTER TO RATS.

TABLE 4.
Body Weight of Rats (Gm.).
Preparatory Period.—Series No. 2 (Duration 52 Days).

Rat No.	Sex.	DATE: 1941.																	
		2/9	10/9	16/9	20/9	23/9	27/9	30/9	3/10	7/10	9/10	11/10	13/10	15/10	17/10	20/10	21/10	22/10	23/10
1	Male.....	44	66	75	87	103	115	117	127	141	147	155	159	162	161	168	169	170	171
2	Male.....	42	55	65	77	92	96	104	114	128	133	140	142	148	153	156	158	158	160
3	Male.....	27	46	58	70	81	93	101	112	128	135	139	141	147	149	152	152	153	154
4	Female.....	31	41	54	66	73	85	92	100	106	112	115	117	120	121	124	125	125	125
5	Female.....	27	36	49	60	63	81	87	95	100	104	107	109	112	113	115	115	117	117
6	Female.....	28	36	46	59	70	80	90	100	106	111	114	114	119	116	119	117	117	118
Average.	Male.....	38	56	66	78	92	101	107	118	132	138	145	147	152	154	159	160	160	161
	Female.....	32	38	50	62	69	82	90	98	104	109	112	113	117	116	119	119	120	120
7	Male.....	44	64	75	90	106	125	131	142	155	158	160	162	169	172	175	178	178	178
8	Male.....	37	52	65	77	90	98	105	120	138	142	150	153	159	159	165	169	168	168
9	Male.....	30	45	56	66	75	85	96	104	112	120	126	126	133	136	141	142	141	142
10	Female.....	30	47	48	61	65	81	92	100	107	111	116	118	123	124	129	131	129	129
11	Female.....	27	43	53	61	68	77	86	92	103	108	111	114	119	120	124	126	127	127
12	Female.....	28	44	58	67	70	85	92	101	110	113	117	118	120	123	124	123	125	125
Average.	Male.....	37	54	65	78	90	103	111	122	135	140	145	147	153	159	160	163	162	163
	Female.....	28	41	53	63	68	81	90	98	107	111	115	117	121	122	126	127	127	127
13	Male.....	50	70	86	105	115	125	—	145	153	—	—	—	178	—	198	199	203	203
14	Male.....	30	47	58	68	79	96	104	113	126	129	134	140	145	145	152	153	155	155
15	Male.....	31	52	61	69	82	96	105	115	129	134	140	144	146	149	150	149	150	150
16	Female.....	39	54	63	69	74	88	95	102	109	112	119	119	123	126	130	132	133	134
17	Female.....	26	43	58	66	78	80	92	100	118	112	115	118	120	120	128	128	128	128
18	Female.....	27	38	50	60	69	84	89	96	106	110	112	115	117	117	119	117	120	120
Average.	Male.....	37	56	68	81	92	106	—	124	136	—	—	—	156	—	166	167	169	169
	Female.....	31	45	57	65	74	84	92	99	111	111	115	114	119	121	126	126	127	127

TABLE 5.
Body Weight of Rats (Gm.).
Curative Period.—Series No. 2 (Duration 21 Days).

Rat No.	Sex.	DATE: 1941.												Remarks.				
		23/10.	24/10.	25/10.	27/10.	28/10.	29/10.	31/10.	1/11.	3/11.	5/11.	6/11.	8/11.		10/11.	11/11.	12/11.	13/11.
1 2 3 4 5 6 Average.	Male.....	171	172	172	172	171	171	170	168	168	165	163	164	164	164	163	162	8
	Male.....	160	159	160	162	161	161	162	162	163	162	161	161	160	160	159	160	1
	Male.....	154	154	155	156	156	157	156	156	153	151	149	145	143	143	142	140	12
	Female.....	125	125	126	125	126	126	124	125	124	124	122	122	112	112	112	113	13
	Female.....	117	116	118	118	119	119	120	120	115	116	117	119	119	120	119	120	2
	Female.....	118	117	117	119	119	121	122	122	123	122	121	125	125	123	122	122	5
Average.	Male.....	162	162	162	163	166	163	163	163	161	161	159	156	156	154	154	154	7
	Female.....	119	119	120	121	121	121	122	122	119	120	120	120	122	122	118	118	2
	Male.....	178	179	181	186	187	187	192	194	197	202	204	205	210	207	210	212	32
	Male.....	168	170	171	176	177	179	183	185	190	191	191	192	193	192	194	195	26
	Male.....	142	142	144	147	150	150	154	154	156	157	158	162	166	167	169	168	26
	Female.....	129	129	131	134	136	138	140	141	142	143	144	144	146	145	145	146	16
Average.	Female.....	127	127	130	132	135	137	140	141	142	144	145	145	147	148	147	148	21
	Female.....	125	125	129	133	134	136	142	144	146	149	149	149	151	151	151	152	26
	Male.....	163	164	165	170	171	172	176	178	181	183	184	186	190	189	191	192	28
	Female.....	127	127	130	133	135	137	141	142	143	145	146	146	148	148	149	149	22
	Male.....	203	207	209	211	214	218	224	229	236	239	241	247	251	252	255	259	53
	Female.....	155	157	159	166	167	170	173	175	177	179	180	182	184	185	187	187	32
Average.	Male.....	150	152	155	162	163	164	169	173	177	179	179	183	184	184	186	187	36
	Female.....	134	133	136	139	140	144	145	145	148	148	148	150	150	152	154	153	19
	Female.....	128	130	133	137	138	137	141	142	143	147	147	147	148	148	149	153	23
	Female.....	120	122	127	135	136	139	142	143	146	147	149	149	151	151	153	153	23
	Male.....	169	172	181	180	181	184	189	192	197	199	200	204	206	207	209	212	40
	Female.....	127	128	132	137	138	140	143	144	146	147	148	149	150	151	152	153	22

TABLE 5 (a).
Body Weight of Rats (Gm.).
Continuation of Curative Period (to 35th day).—Series No. 2.

Rat No.	Sex.	DATE : 1941.										REMARKS.
		15/11	17/11	19/11	20/11	22/11	24/11	25/11	26/11	27/11	27/11	
Control Group D.	1	165	173	168	170	174	182	185	187	187	187	Rat cured; increase in body weight 25 grms. (14 days).
	2	157	160	180	187	196	204	209	208	208	210	Rat cured; increase in body weight 50 grms. (14 days).
	5	124	130	135	137	141	145	147	147	147	150	Rat cured; increase in body weight 31 grms. (14 days).
	6	122	134	139	143	149	158	160	160	159	160	Rat cured; increase in body weight 38 grms. (14 days).
	7	213	216	217	220	223	227	228	228	228	231	Total gain (35 days) = ± 53 grms. Killed for Liver Vit. A. Assays, 28/11/41.
	8	197	200	202	205	209	213	215	216	216	218	Total gain (35 days) = ± 50 grms.
Group E.	12	152	153	155	155	155	158	159	159	160	160	Total gain (35 days) = ± 35 grms. Killed for Liver Vit. A. Assays, 28/11/41.
	10	146	149	149	150	153	154	155	154	155	155	Total gain (35 days) = ± 26 grms.
	13	262	269	271	276	279	284	285	285	285	289	Total gain (35 days) = ± 85 grms. Killed for Liver Vit. A. Assays, 28/11/41.
Group F.	14	190	193	195	198	198	200	203	205	207	207	Total gain (35 days) = ± 52 grms.
	18	158	159	159	160	164	165	166	167	169	169	Total gain (35 days) = ± 49 grms.
	17	152	157	157	157	159	159	160	160	160	161	Total gain (35 days) = ± 33 grms.

TABLE 6.
Liver Assays for Vitamin A Reserves.
Rats in Series No. 2.

Rat No.	Sex.	PREPARATORY PERIOD.						CURATIVE PERIOD.									
		Initial Stage.			End Stage.			After 21 days.			After 35 days.						
		Wht.	Liver Wht.	Vit. A. I.U.	Stor. age I.U.	Wht.	Liver Wht.	Vit. A. I.U.	Stor. age I.U.	Wht.	Liver Wht.	Vit. A. I.U.	Stor. age I.U.	Wht.	Liver Wht.	Vit. A. I.U.	Stor. age I.U.
			Gms.		Gms.		Gms.		Gms.		Gms.		Gms.		Gms.		Gms.
—	Male.....	50	2.3	10.8	10.8	—	—	—	—	—	—	—	—	—	—	—	—
—	Female.....	48	2.1	8.4	8.4	160	8.0	—	—	—	—	—	—	—	—	—	—
—	Male.....	—	—	—	—	125	6.5	0	—	—	—	—	—	—	—	—	—
—	Female.....	—	—	—	—	—	—	0	—	—	—	—	—	—	—	—	—
3	Male.....	—	—	—	—	—	—	—	—	142	4.4	0	0	—	—	—	—
4	Female.....	—	—	—	—	—	—	—	—	112	4.1	0	0	—	—	—	—
9	Male.....	—	—	—	—	—	—	—	—	168	7.6	0	0	—	—	—	—
11	Female.....	—	—	—	—	—	—	—	—	148	7.4	0	0	—	—	—	—
15	Male.....	—	—	—	—	—	—	—	—	186	9.0	1.2	1.2	—	—	—	—
16	Female.....	—	—	—	—	—	—	—	—	153	7.5	0.6	0.6	—	—	—	—
7	Male.....	—	—	—	—	—	—	—	—	—	—	—	—	231	10.8	0	0
12	Female.....	—	—	—	—	—	—	—	—	—	—	—	—	160	6.7	0	0
13	Male.....	—	—	—	—	—	—	—	—	—	—	—	—	289	13.1	0.6	0.6
18	Female.....	—	—	—	—	—	—	—	—	—	—	—	—	169	8.6	3.6	3.6