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The Effect of Intermittent Starvation on Calcification, Food Utilization, and Tissue Composition.

By J. H. KELLERMANN, Section of Biochemistry, Onderstepoort.

THE primary function of livestock is to convert vegetable into animal products such as meat, milk, wool, etc., but animals of the same breed, sex, age and size often vary greatly in ability to convert their food into the various animal products.

The extensive experiments of Palmer and Kennedy (1931) with rats showed that a major, if not the controlling cause of individual variation in gain in weight of animals on the same diet is due to individual variation in efficiency of food utilization. Similar results were obtained by Winters (1936). He fed steers individually for three years and showed clearly that the variation in efficiency of feed utilization between steers of a given market grade was sufficient to be of practical importance in livestock production. Under the experimental conditions it cost 26 to 44 per cent. more to produce 100 lb. gain in the least efficient than in the most efficient steer. This quality of efficient or inefficient food utilization is, as proved by Morris, Palmer and Kennedy (1933), hereditary, and the problem therefore suggests itself that lines of livestock should be bred which are not only more efficient in making gains but also more uniform in this respect.

It is probable that food utilization and subsequent growth may also be influenced by the method of feeding. For instance, Morgulis (1923) states that " Von Seeland (1887) from his experiments with chickens claims that intermittent brief fasts conduce to greater development of the body. He used birds which already attained a constant body weight, and some of them he fed regularly every day while others were deprived of food from time to time for periods of one to two days. He discovered that the chickens thus periodically fasted became heavier than the control birds although they were actually getting less feed than the latter. According to Von Seeland the increase in weight was not due to deposition of fat, but to an accumulation of protein material, i.e., to an increase in flesh. No valid chemical evidence has been offered for this claim by Von Seeland, who maintains that the periodic fasting had the effect of making the body heavier, stronger and more solid ".

Robertson, Marston and Walters (1934) subjected mice to fasts of two days out of every seven. While the immediate effect of the intermittent starvation was to reduce the growth rate of the mice, the mean body weight of the animals so treated sooner or later surpassed that of the controls, which continually received a superabundance of the same food. This effect was especially noticeable in males. The authors state: " While the periodic abstinence from food did not significantly increase the life duration, it is remarkable that such treatment certainly did not decrease the expectancy of life, and, furthermore, the significant increase in the body weight of the male animals which followed such treatment is most striking when we consider the relatively high rate of energy consumption of the small rodent ". Unfortunately these workers did not measure the food intake of their experimental animals. Because growth is normally related to the amount of food consumed, a record of the latter might have helped to explain the significantly greater increase in weight of the fasted male rats as compared with the gain of their control mates. Morgulis (1912, 1913) found that intermittently fasting salamanders (*Triton cristatus*) reached somewhat more than two-thirds of the body weight of the continually fed animals although the fasted salamanders actually consumed only about one-half the amount of food consumed by the controls.

In view of the lack of data on the food utilization and body composition of the control and fasted animals in the above experiments, no explanation could of course be advanced for the difference in gain of the respective groups. The following experiments were therefore planned with the object of collecting more information on these questions.

Experimental.

Experiment 1—Bone Calcification.

Albino rats of about three to four weeks old and of the London Strain of the Wistar Institute stock were used in this experiment. Each rat was kept in a separate cage with a raised screen bottom. The paired-feeding method of Mitchell and Beadles (1930) was employed, and all pairs were " isogenic ", that is, they were of the same sex, litter and as nearly as possible of the same weight. There were eleven pairs of rats in this experiment.

The composition of the ration fed is given in Table I. The Ca and P contents of the ration were 0·62 and 0·44 per cent. with a Ca:P ratio of 1·4:1. The total protein content ($N \times 6 \cdot 25$) was 22·4 per cent. Both rats in each pair received the same ration except that one animal was fasted two successive days out of every seven. Each fast was followed by five days of normal feeding. The food intake was so regulated that the total food consumption for each week was the same for the control rat and its fasted pair mate. The animals were weighed once weekly and just prior to every two-day fast. Distilled water was always available to all the animals.

TABLE I.

Composition of Ration in Percentage by Weight.

Yellow maize meal	60
Linseed oil meal	6
Skim milk powder	6
Crude casein	10
Dried brewers' yeast	5
Lucerne meal	3
Butter fat	5
Beef liver (dried at 70° C.)	2
Bone ash	1
Cod-liver oil	1
$CaCo_3$	0·5
NaCl	0·5

At the end of five weeks the rats were killed and the femurs removed for the determination of ash. They were dissected free from soft tissues and extracted according to the method as described by Steenbock and co-workers (1930) with hot 96 per cent. alcohol with frequent change of alcohol, in a Soxhlet apparatus for 5 days. The femurs were then dried in an electric oven at 105° C. and ashed. The percentage of ash was calculated on the dry-fat-free basis. The results of these analyses together with the gain and food consumption of the rats are given in Table II. These results were subjected to statistical analysis* and the findings presented in Tables III, IV, V and VI.

TABLE II.

Growth and Bone Calcification as Affected by Starvation.

Pair No. and Sex.	Treatment.	Total Food.	Initial Weight.	Gain.	Weight of Femur.	Weight of Ash.	Percentage of Ash.
		g.	g.	g.	g.	g.	
1 ♂	Control	345	56	91	·2417	·1437	59·47
	Fasted	345	57	97	·2325	·1358	58·42
2 ♂	Control	376	59	103	·2607	·1534	58·83
	Fasted	376	61	106	·2477	·1449	59·20
3 ♂	Control	381	63	99	·2562	·1532	59·78
	Fasted	381	64	92	·2205	·1298	58·86
4 ♂	Control	393	64	95	·2552	·1531	59·99
	Fasted	393	65	103	·2501	·1477	59·06
5 ♂	Control	341	69	73	·2429	·1479	60·90
	Fasted	341	69	79	·2306	·1365	59·20
6 ♂	Control	396	59	89	·2387	·1366	57·22
	Fasted	396	60	106	·2398	·1419	59·18
7 ♀	Control	334	56	62	·2115	·1250	59·12
	Fasted	334	56	67	·1994	·1155	57·93
8 ♀	Control	334	63	67	·2145	·1293	60·30
	Fasted	332	63	68	·2154	·1304	60·57
9 ♀	Control	360	75	77	·2644	·1627	61·56
	Fasted	356	72	71	·2278	·1407	61·85
10 ♀	Control	383	58	79	·2255	·1324	58·70
	Fasted	383	58	85	·2119	·1268	59·84
11 ♀	Control	347	56	75	·2294	·1397	60·89
	Fasted	345	58	77	·2196	·1315	59·89

* The results of all the experiments described in this paper were analysed according to the analysis of variance and covariance technique as set out by Fisher (1936).

Table III.

Table of Means—Gain in Weight.

	Males.	Females.	Difference.	S.E. Difference.	Percentage Difference.	t.	Significance.
Unadjusted	94·42	72·80	21·61	5·21	29·70	4·15	P. = ·01
Adjusted for initial weight	94·54	72·66	21·88	4·71	30·11	4·65	P. = ·01
Adjusted for total food . .	91·60	76·21	15·39	4·02	20·19	3·83	P. = ·01
	Control.	**Fasted.**	**Difference.**	**S.E. Difference.**	**Percentage Difference.**	**t.**	**Significance.**
Unadjusted	82·72	86·45	3·72	1·987	4·5065	1·8758	None.
Adjusted for initial weight	83·15	86·03	2·88	2·214	3·4636	1·3008	None.

Table IV.

Table of Means.—Percentage Ash.

Control.	Fasted.	Difference.	Percentage Difference.	S.E. Difference.	t.	Significance.
59·703	59·442	0·261	0·44	0·3553	0·735	None.
Male.	**Female.**	**Difference.**	**Percentage Difference.**	**S.E. Difference.**	**t.**	**Significance.**
59·177	60·047	0·870	1·47	0·5603	1·553	None.

Table V.

Table of Means.—Weight of Femur.

	Males.	Females.	Difference.	S.E. Difference.	Percentage Difference.	t.	Significance.
Unadjusted...........	0·2428	0·2219	0·0209	0·007,044	9·42	2·9671	P. = ·05
Adjusted for initial weight..............	0·2425	0·2223	0·0202	0·006,029	9·09	3·3505	P. = ·05
Adjusted for gain in weight..............	0·2365	0·2294	0·0071	0·011,250	3·10	0·6311	None.

	Control.	Fasted.	Difference.	S.E. Difference.	Percentage Difference.	t.	Significance.
Unadjusted...........	0·2401	0·2266	0·0135	0·003,962	5·96	3·4074	P. = ·01
Adjusted for initial weight..............	0·2412	0·2255	0·0157	0·003,763	6·96	4·1722	P. = ·01
Adjusted for gain in weight..............	0·2430	0·2237	0·0193	0·002,725	8·63	7·0826	P. = ·01

Table VI.

Table of Means.—Weight of Ash.

	Control.	Fasted.	Difference.	S.E. Difference.	Percentage Difference.	t.	Significance.
Unadjusted...........	·143,364	·134,682	·008,682	·002,686	6·446	3·2323	P. = ·01
Adjusted for femur weight..............	·1390	·1390	0	·001,240	0	0	None.

	Male.	Female.	Difference.	S.E. Difference.	Percentage Difference.	t.	Significance.
Unadjusted...........	0·1437	0·1334	0·0103	0·00517	7·72	1·992	None.
Adjusted for femur weight..............	0·1368	0·1416	− 0·0048	0·00143	3·44	3·342	P. = ·05

From the table giving the mean gain in weight, it is clear that in general the males gained significantly more than the females, the probability P being 0·01. This difference remained significant even when the initial weights and total food intakes were equalised for the males and females by means of the analysis of covariance technique. However, no significant difference was found in the gain in weight between the control and fasted animals. Likewise, there was found to be no significant difference in the percentage ash (Table IV) of femurs of male versus female or control versus fasted animals. On the other hand, a comparison of the weights of the femurs (Table V) shows that the bones of the males were significantly heavier than those of the females (P = ·05) but the significance disappeared when the femur weights were adjusted for gain in weight. Furthermore, it is of special interest that even five days after the last two-day fast, and notwithstanding an equalised weekly consumption of the same ration, the femur weights of the control animals were significantly heavier (P = ·01) than those of the fasted rats. This difference remained significant even when the initial weights or gain in weight of the control rats were made equal to those of the fasted animals. It would seem therefore that the fasting caused some disturbance in the process of calcification which manifested itself in the form of lighter bones.

The results in Table VI show that the ash means of the control versus fasted rats was significant at P = ·01, but when weight of ash is adjusted for weight of femur this significant difference disappears. This is according to expectation for it seems reasonable that the lighter femur, caused by fasting, should have less ash. It is in fact a verification of the result obtained when the percentage ash data were analysed, namely, that the difference between the percentage femur ash of the control versus fasted rats was insignificant.

The male versus female ash means show no significant difference. On adjustment for femur weights, a significant difference does emerge, but in view of the fact that this significance is not supported by an analysis of the percentage ash data, it is not wise to attempt an explanation at this juncture.

Experiment 2.—Food Utilization.

White rats of about three to four weeks of age were used in this experiment. Each animal was kept in a separate cage with a raised screen bottom. In addition to distilled water to which the rats had always free access they were fed *ad libitum* a ration the composition of which is given in Table I. The total food consumption for each animal was recorded. The rats were weighed once weekly.

After the animals had been fed the ration for four weeks, their efficiency quotients were calculated according to the method of Palmer and Kennedy (1929).* They were then paired with respect to sex and efficiency of food utilization and fed the same ration and distilled water for another sixteen weeks when they were about 5½ months of age but not yet fully grown. There were thirteen pairs and one animal out of every pair was fasted for two days out of every seven as described previously. In the case of seven pairs only

* The dry matter only, not the digestible dry matter, was used in these calculations.

the total food intake for each animal was recorded (*ad libitum* feeding) whereas the food intake of the remaining pairs was so regulated that the total food consumption for each week was the same for the control rat as that of its fasted pair mate (equalised food intake). The efficiency quotients were recalculated for the last sixteen weeks. The results obtained with the rats fed *ad libitum* are tabulated in Table VII. The findings of a statistical analysis of these results are presented in Tables VIII, IX and X.

TABLE VII.

Food Utilization as affected by Starvation (Ad libitum food consumption).

Pair No. and Sex.	Preliminary E.Q.	Treatment.	Initial Weight.	Gain.	Mean Weight.	Total Food.	Experimental E.Q.
			g.	g.	g.	g.	
1 ♂	2·54	Control	182	285	335·4	1,887	1·97
	2·45	Fasted	180	134	254·4	1,277	3·75
2 ♂	2·34	Control	191	250	347·8	1,834	2·11
	2·34	Fasted	193	154	296·6	1,526	3·34
3 ♂	2·24	Control	195	187	307·8	1,484	2·58
	2·25	Fasted	198	167	301·7	1,541	3·06
4 ♀	3·76	Control	143	107	213·9	1,337	5·84
	3·77	Fasted	148	95	210·6	1,265	6·32
5 ♀	3·94	Control	139	127	217·8	1,503	5·43
	3·96	Fasted	131	98	195·9	1,149	5·98
6 ♀	4·02	Control	133	109	197·0	1,264	5·89
	4·05	Fasted	141	95	196·1	1,195	6·41
7 ♀	4·31	Control	129	93	185·1	1,180	6·85
	4·41	Fasted	130	108	194·8	1,170	5·56

TABLE VIII.

Table of Means.—Gain in Weight on ad libitum Food Consumption.

Males.	Females.	Difference.	S.E. Difference.	Percentage Difference.	t.	Significance.
196·2	104·0	92·2	8·8846	88·65	10·374	P. = ·01

Control.	Fasted.	Difference.	S.E. Difference.	Percentage Difference.	t.	Significance.
165·4	121·6	43·8	16·61	36·0	2·64	P. = ·05

Table IX.

Table of Means.—Total Food Intake.

Males.	Females.	Difference.	S.E. Difference.	Percentage Difference.	t.	Significance.
1591·5	1257·9	333·6	57·5	26·52	5·802	P. = ·01

Control.	Fasted.	Difference.	S.E. Difference.	Percentage Difference.	t.	Significance.
1498·4	1303·3	195·1	91·76	14·97	2·126	None.

Table X.

Table of Means—Efficiency Quotients.

Females.	Males.	Difference.	S.E. Difference.	Percentage Difference.	t.	Significance.
6·04	2·80	3·24	0·14	115·71	23·14	P. = ·01

Control.	Fasted.	Difference.	S.E. Difference.	Percentage Difference.	t.	Significance.
4·38	4·92	0·54	0·31	10·98	1·74	None.

From these tables it is clear that in general the males gained significantly more than the females. Because the food intake of the males was significantly greater than that of the females (Table IX) one might have considered this factor as the major cause of the difference in gain. However, the statistical findings show that the significant difference (P = ·01) between the efficiency indices of males and females (Table X) in this experiment also played an important part in bringing about the different rates of gain.

The control rats gained significantly more than their fasted mates. The food intake of control rats was not significantly greater than that of the fasted rats. The percentage difference, however, was 15 per cent. (see Table IX) and it seems reasonable that this was the cause of the different gains in weight.

The statistical findings obtained by analysing the data (Table XI) given by the pair mates whose food intake was equalised are presented in Tables XII, XIII, and XIV.

TABLE XI.

Food Utilization as Affected by Starvation (Equalised Food Consumption).

Pair No. and Sex.	Preliminary E.Q.	Treatment.	Initial Weight.	Gain.	Mean Weight.	Total Food.	Experimental E.Q.
			g.	g.	g.	g.	
1 ♂	2·98	Control	171	160	257·6	1,370	3·32
	2·89	Fasted	177	176	276·6	1,370	2·81
2 ♂	2·64	Control	186	166	288·0	1,460	3·05
	2·48	Fasted	195	157	291·6	1,460	3·19
3 ♂	3·20	Control	165	217	285·6	1,554	2·51
	3·13	Fasted	181	191	291·6	1,554	2·79
4 ♀	4·41	Control	133	79	175·8	1,042	7·50
	4·63	Fasted	137	92	189·2	1,036	5·95
5 ♀	4·71	Control	137	80	194·9	1,071	6·87
	4·64	Fasted	128	87	186·8	1,071	6·59
6 ♀	3·47	Control	153	52	193·1	1,091	10·86
	3·77	Fasted	148	82	202·9	1,090	6·55

TABLE XII.

Table of Means—Gain in Weight on Equalised Food Intake.

Males.	Females.	Difference.	S.E. Difference.	Percentage Difference.	t.	Significance.
177·8	78·7	99·1	14·46	125·9	6·85	P. = ·01

Control.	Fasted.	Difference.	S.E. Difference.	Percentage Difference.	t.	Significance.
125·7	130·8	5·1	7·0046	4·06	0·73	None.

TABLE XIII.

Table of Means—Total Food Intake.

Males.	Females.	Difference.	S.E. Difference.	Percentage Difference.	t.	Significance.
1461·3	1066·8	394·5	55·21	36·98	7·145	P. = ·01

TABLE XIV.

Table of Means—Efficiency Quotients.

Females.	Males.	Difference.	S.E. Difference.	Percentage Difference.	t.	Significance.
7·39	2·95	4·44	0·68	150·51	6·53	P. = ·01

Control.	Fasted.	Difference.	S.E. Difference.	Percentage Difference.	t.	Significance.
5·69	4·65	1·04	·6071	22·37	1·71	None.

From Tables XII and XIII it is evident that the males consumed significantly more food than the females and also made much better gains (P. = ·01). From the mean efficiency quotients given in Table XIV it is clear that the males again utilized their food significantly better (see also Table X) than the females which substantiates the results of Palmer and Kennedy (1931), Morris, Palmer and Kennedy (1933) and Kellermann (1938, 1), (1938, 2), (1938, 3). However, no differences were found between the gain in weight, food intake or efficiency of food utilization of the control animals and the respective values of the fasted rats.

Taking the results on food utilization then as a whole one is led to the conclusion that, in the rat and under the experimental conditions, short periods of abstention from food had, *per se*, no beneficial effect on food utilization and subsequent growth.

Tissue Composition.

At the conclusion of the experiment and five days after the last fast the seven pairs of control and fasted animals that were fed *ad libitum* (see Table VII) were killed by a blow on the head. Their livers, thigh and back muscles (on each side of spine) were dissected for analysis.

The livers and the muscle tissues of the control rats were pooled separately and finely ground by passing them several times through a small meat mincer. The tissues of the fasted animals were treated similarly and appropriate samples immediately weighed out for the determination of water, ash, protein and fat. The chemical determinations were made according to the methods of the Association of Official Agricultural Chemists (1935). The water was determined in a Heinz standard ground vacuum apparatus. The apparatus was run under high vacuum for 20 hours at ca. 20 mm. Hg. and the temperature never exceeding 40° C. The ash was determined by ignition to a dull redness in an electric furnace, fat by extraction with ether, and the nitrogen by the Kjeldahl method. The protein was calculated from the total nitrogen by use of the factor 6·25. The results are presented in Table XV.

TABLE XV.

The Composition of Fresh Tissues of Control and Fasted Rats.

Percentage.	Control Rats.		Fasted Rats.	
	Liver.	Muscle.	Liver.	Muscle.
Water	67·74	66·71	67·84	67·67
Protein	21·11	20·87	21·51	20·61
Fat	3·90	12·31	3·68	10·07
Ash	1·41	1·10	1·39	1·15

From a comparision of the values of the control rats with those of the fasted ones, it is evident that the periodical short fasts had no appreciable effect on the composition of the liver or muscle tissues. These results are therefore in support of the work of Lee and Lewis (1934) who also give a good review of the literature on the effects of fasting on the composition of tissues.

SUMMARY.

1. Data are presented on the effects of short intermittent fasts on bone calcification, food utilization and tissue composition.

2. No significant differences were found between the percentage ash of femurs of males *versus* females or control *versus* fasted animals.

3. No significant differences were found between the control and fasted animals with respect to food utilization but the males made uniformly better use of their food than the females.

4. Intermittent periods of fasting had no effect on the composition of liver and muscle tissues.

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