XXXIV. A STUDY OF RATS ON A NORMAL DIET IRRADIATED DAILY BY THE MERCURY VAPOUR QUARTZ LAMP OR KEPT IN DARKNESS.

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HISTORICAL.

IN the course of a study on the absence of rickets during confinement on a good diet, Mellanby [1921] kept four puppies in small kennels in the dark for a period of thirteen weeks. Their diets consisted of:

- (a) bread, milk and meat;
- (b) bread, milk, meat and bone;
- (c) bread, milk, meat and bone that had been autoclaved at 120° for 15 minutes.

The puppies were ten weeks old at the beginning of the experiment and were killed at the age of 26 weeks. Histologically, no sign of rickets was found. Chemical analysis of the shafts of the femurs gave the following percentages of CaO in the dry bone:

Puppy No. 25	...	...	42.92
„ 26	...	...	38.13
„ 27	...	...	37.46
„ 28	...	...	30.00

These values Mellanby considers to be high. It is evident from the foregoing facts that the calcium content of the bones was unaffected by 13 weeks of confinement in the dark.

Raczynsky [1912] investigated the effect of sunlight and darkness on puppies during the period of lactation. He took two puppies from the same litter, born in May, kept one in total darkness from birth and the other in sunlight, both suckled by the same mother. After six weeks they were killed

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and the bodies chemically analysed. The result is given in the following table:

	Per 100 g. of body weight	
	Puppy in sunlight	Puppy in darkness
	g.	g.
CaO	1.58	0.98
P ₂ O ₅	1.19	0.86
MgO	0.05	0.04
Cl	0.16	0.35
Fe	0.02	0.02

From the fact that the calcium and phosphorus content of the puppy kept in the dark was lower than that of the one kept in sunlight, he surmised that the former had rickets. The diet of the mother is not known, therefore it cannot safely be assumed that the puppies received an optimal amount of calcium, phosphorus and anti-rachitic organic factor. Comparing the results with those about to be recorded, one ventures to suggest that the mother's diet probably was not complete.

EXPERIMENTAL.

Radiation. Since sunlight was not available, the radiations from a mercury vapour quartz lamp were used as a substitute. The lamp employed is a Pistolette, manufactured by the Hewittic Electric Co. The shortest wave length of its spectrum is 230μ and a daily twenty minute exposure to the rays from this lamp has been found to prevent or cure rickets in rats on diet No. 3143 of McCollum and his collaborators [1921], and on other rickets-producing diets.

Two litters of rats were divided into two groups with an equal number of the same sex in each. One group was kept in total darkness from the time the rats were weaned, while the other was kept in a room with a large double window consisting of plate glass about half-an-inch thick, through which daylight had to filter to enter the room. But the latter group was exposed for 20 minutes daily at a distance of 60 cm. to the radiations from the mercury vapour quartz lamp.

Diets. In the case of litter XXI, both groups received the following diet:

<i>Diet N 1</i>	Inactivated commercial "casein"	...	20.0 g.
	Starch (wheat)	...	50.0
	Cotton-seed oil (hardened)	...	10.0
	Cod-liver oil	...	5.0
	Salt mixture (McCollum No. 185)	...	5.0
	Calcium carbonate	...	3.1
	Marmite	...	5.0
	Lemon juice	...	5.0 cc.
	Distilled water	...	46.9 cc.

In the above diet the percentage of calcium in the paste was 1.07, of phosphorus 0.53 and of cod-liver oil 3.66, all optimal amounts for these substances.

The two groups of litter XXII were subdivided so that half the rats in each group (darkness and light) received the same diet as litter XXI and the other half a similar diet in which butter was substituted for both the cod-liver and cotton-seed oils. This diet consisted of:

<i>Diet N 2</i>	Inactivated commercial "casein"	...	20.0 g.
	Starch (wheat)		50.0
	Fresh butter		15.0
	Salt mixture (No. 185)		5.0
	Calcium carbonate		3.1
	Marmite		5.0
	Lemon juice		5.0 cc.
	Distilled water		46.9 cc.

The rats of litter XXI were 23 days and those of litter XXII 28 days old at the beginning of the experiment. At the end of eight weeks both groups were killed. That experimental period was chosen as it was the one used most frequently in other investigations and because the rats were then about twelve weeks old and therefore nearly full-grown.

Table I.

No. of rat	Sex	No. of litter	Diet	Age (in days)		Days on diet	Average amount of food eaten daily	Weight grams		Gain grams	In bones		
				Initial	Final			Initial	Final		% H ₂ O	% Ca wet	% Ca dry
G. 409	♀	XXI	N 1	23	79	56	12.6	28	101	73	40.95	12.51	21.21
G. 410	♂	XXI	N 1	23	79	56	17.2	34	172	138	40.10	12.67	21.15
G. 411	♂	XXI	N 1	23	79	56	16.0	38	161	123	40.88	12.51	21.13
Averages											40.64	12.56	21.16
G. 412	♀	XXI	N 1	23	79	56	12.2	33	113	80	38.25	12.80	20.73
G. 413	♂	XXI	N 1	23	79	56	15.0	33	139	106	38.12	12.72	20.55
G. 414	♂	XXI	N 1	23	79	56	17.5	38	160	122	42.28	12.22	21.15
Averages											39.55	12.58	20.81
G. 424	♀	XXII	N 1	28	84	56	14.5	31	123	92	41.46	13.05	22.28
G. 425	♂	XXII	N 1	28	84	56	21.1	36	198	162	40.00	13.61	22.67
Averages											40.73	13.33	22.47
G. 426	♀	XXII	N 1	28	84	56	14.6	27	131	104	38.95	13.69	22.68
G. 427	♂	XXII	N 1	28	84	56	17.7	34	191	157	39.85	13.28	22.07
Averages											39.40	13.48	22.37
G. 428	♀	XXII	N 2	28	84	56	22.0	32	142	110	37.14	14.19	22.58
G. 429	♂	XXII	N 2	28	84	56	19.8	31	178	147	39.65	13.84	22.95
Averages											38.38	14.01	22.76
G. 430	♀	XXII	N 2	28	84	56	16.5	29	136	107	35.85	14.56	22.70
G. 431	♂	XXII	N 2	28	84	56	18.5	33	193	160	38.83	14.28	23.34
Averages											37.34	14.42	23.02

NOTE. The histological diagnosis in each rat is normal.

Table II.

All rats on normal diets	Average % H ₂ O	Average % Ca (wet bones)	Average % Ca (dry bones)
In darkness	40.03	13.30	22.13
In daylight + ultra-violet	38.88	13.49	22.06

In life there was no noticeable difference between the two groups. The growth curves of both males and females exceeded greatly the Donaldson

Rat G. 431. "Butter" Diet in Darkness. Age 84 days. Weight 160 grams. Rib.

Co. Cortical bone, thick and well calcified.

O. Physiological osteoid

Sp. Primary spongiosa. Trabeculae numerous, thick, straight and well calcified.

Z.P.C. Zone of provisional calcification well calcified.

H.C. Hypertrophic cartilage cells.

Z.C.P.C. Zone of proliferating cartilage cells.

R.C. Resting cartilage.

B.M. Bone marrow.

Diagnosis: Normal rib. Haem. and Eosin. Magn. $\times 40$.

Note: The ribs of all the rats were similar to the one illustrated above.

curve of normal growth for the same period of life. The rats living in darkness grew as quickly and reached as high an average maximum weight as those kept in daylight and exposed daily to the mercury vapour quartz lamp.

At autopsy there were no gross differences between the two groups. The eyes were normal and the teeth light yellow and transparent. All the rats were well nourished with an abundant deposit of fat in the natural regions. The musculature was well developed and of good colour. The chest was well developed, and there was no macroscopical abnormality in the ribs or long bones.

Histologically, the bones were normal in every respect, those of the two groups showing practically no differences (see Table I and Pl. IV).

The chemical analysis of the hind legs, carried out by Aron's method [1910], showed a very close agreement between the two groups. In no case was the variation in the percentage of calcium and of water in the bones of these groups greater than that found normally among rats from the same litter (see Table I).

The individual (Table I) and average (Table II) results are remarkably close for the rats kept in darkness and those that were kept in the light and in addition were irradiated daily with the mercury vapour quartz lamp.

SUMMARY.

Two litters of rats were placed on normal diets containing an optimal amount of calcium, phosphorus and the fat-soluble organic factor; in the diet of one litter this last was supplied by cod-liver oil, while for the other, two diets, one containing butter and the other cod-liver oil were used.

One-half of each litter was kept in the dark and the other in daylight with the addition of a daily irradiation for 20 minutes under a mercury vapour quartz lamp.

No appreciable difference was found between the growth of the two groups or histology and calcium content of the bones.

CONCLUSIONS.

Rats on a normal diet containing an optimal quantity of calcium, phosphorus, and fat-soluble organic factor, whether kept in darkness or in daylight supplemented by irradiation from a mercury vapour quartz lamp, grow equally well, and the development and calcification of their bones are normal.

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REFERENCES.

- Aron, H. and Sebaauer, R. (1910). *Abderhalden's Handbuch der biochemischen Arbeitsmethoden*, 1, 413.
 McCollum, Simmonds, Shipley and Park (1921). *J. Biol. Chem.* 47, 507.
 Mellanby, E. (1921). *Experimental Rickets, Medical Research Council Special Report Series*, No. 61.
 Raczyński, J. (1912). *Compt. Rend. Assoc. Internat. Pédiatrie*, 308. Quoted by Park, *Phys. Rev.* 1923, 3, 106.