LXXIX. THE SUPPLEMENTARY VALUE OF LIGHT RAYS TO A DIET GRADED IN ITS CONTENT OF FAT-SOLUBLE ORGANIC FACTOR.

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INTRODUCTION.

HUME [1922], and the authors [1922] showed that rats on a diet very deficient in the fat-soluble growth-promoting factor, if they are irradiated for about ten minutes daily by the mercury-vapour quartz lamp, grow much better than non-irradiated rats on the same diet. But they do not go on growing normally; eventually growth fails, and the subsequent history is that of non-irradiated rats on a — *A* diet. This led to the conclusion that light rays promote growth by enhancing and prolonging in some way the action of the fat-soluble *A* which is stored in the body of animals at the time they are put on the — *A* diet. Now some rats on the — *A* diet employed (diet *F* of this investigation) develop experimental rickets [Korenchevsky, 1921; Goldblatt, 1923]. But histological examination of the bones of many irradiated rats on the same — *A* diet revealed no evidence of rickets¹. Most of them were practically normal, the only pathological change observed in some being slight or moderate osteoporosis. Chemical examination of the bones of these irradiated rats showed a much higher percentage of calcium than in the control rats, but lower than that of normal rats on a complete diet¹. Recently one of the authors [Goldblatt, 1923] showed that when rats are fed on diets graded in their content of fat-soluble organic factor (from the optimal amount to practically complete absence of it) there results a corresponding gradation in the growth of the animals and in the degree of calcification of their bones. The present study was planned in order to determine the minimal amount of fat-soluble organic factor which, supplemented by light rays, can effect the normal growth of rats as well as the normal development and calcification of their bones.

¹ Unpublished.

EXPERIMENTAL.

In this investigation the same six diets were employed as in the study of the effect of grading the quantity of fat-soluble organic factor in a diet upon the development and calcification of the bones of rats [Goldblatt, 1923]. The basal diet, of which the six diets employed were variations, consisted of

Commercial casein	...	...	...	...	20
Starch (wheat)...	...	...	...	...	50
Cotton-seed oil	...	...	...	...	15
Salt mixture (McCollum and Davis, No. 185)					5
Marmite	...	...	...	...	5
Lemon-juice	...	...	...	...	5
Distilled water	...	...	...	...	50

When the casein employed is the unpurified commercial product, it supplies to the above diet an amount of fat-soluble organic factor which will promote about 66 % of the growth of a rat on the same diet, but with sufficient cod-liver oil added to supply an optimal amount of the organic factor. The diet containing 20 % of unpurified casein as the sole source of fat-soluble organic factor will be referred to as diet *B*. An attempt was also made to determine the exact amount of fat-soluble growth-promoting factor in this diet. Rats were put on a diet completely deficient in fat-soluble *A* (diet *F* of this investigation), and when they had ceased growing for three weeks were changed to *B* diet and in addition varying amounts of cod-liver oil were administered daily. Of this oil, 2.25 mg. daily were known to promote practically normal growth for four weeks in a rat that had ceased growing on the — *A* diet (diet *F*). When *B* diet was substituted, the addition of only 0.56 mg. of oil a day was necessary to promote the same amount of growth in four weeks. Thus the fat-soluble *A* in the remaining 1.69 mg. must have been supplied by diet *B*. A closer titration was not attempted. Thus it would seem that diet *B* contains approximately 75 % of the amount of fat-soluble *A* necessary to promote normal growth. This agrees rather well with the previous statement that diet *B* is able to promote about two-thirds of the normal growth of rats during a period of eight weeks.

In the previous investigation 3 % of crude cod-liver oil was added to diet *B*, and this resulted in a diet which contained an amount of fat-soluble organic factor considerably in excess of the optimal. This diet was called *AA* and considered the normal control diet. In the present investigation, instead of incorporating the cod-liver oil with the diet, the normal control rats were kept on *B* diet and 200 mg. of crude cod-liver oil were administered by pipette daily to the rats. This will also be referred to as diet *AA*. This is approximately half the amount of cod-liver oil which the rats on diet *AA* in the former study received, but is also far in excess of the minimal amount necessary to promote normal growth. When tested by the biological method of Zilva and Miura

[1921] 2.25 mg. daily of the oil employed gave a positive result, *i.e.* was able to promote normal growth for four weeks.

By replacing various amounts of the unpurified casein in diet *B* by casein inactivated by heat and oxidation, the amount of fat-soluble organic factor was reduced. Diets *C*, *D*, *E* and *F* were thus made, which differed from *B* only in the quantity of organic factor they contained. The following table illustrates the source and quantity of the fat-soluble organic factor in the six diets.

Table I.

Diet	Basal diet containing		
	Unpurified casein	Purified casein	Cod-liver oil
<i>AA</i>	20	0	200 mg. daily
<i>B</i>	20	0	0
<i>C</i>	15	5	0
<i>D</i>	10	10	0
<i>E</i>	5	15	0
<i>F</i>	0	20	0

Thus, considering that diet *B*, in its 20 % of unpurified casein, contains about 70 % of the minimal amount of fat-soluble *A* which will promote normal growth, it follows that diet *C* contained 52.5 %, *D* 35 %, *E* 17.5 % and *F* none at all.

Forty-eight rats (four litters of twelve¹) were employed in this investigation. From every litter two rats were put on every one of the six diets, one being irradiated for ten minutes daily at a distance of 60 cm. by the mercury-vapour quartz lamp. Thus there were four rats on every one of the six diets (a total of twenty-four) which were irradiated daily, and the same number on the same diets who were kept in a well-ventilated room with a large double window of plate glass half an inch thick through which light had to filter. Except during the time the irradiated rats were under the lamp, they were kept in the same room as the non-irradiated controls.

Growth.

The average final weight and average maximum gain in weight of the non-irradiated rats on the six diets showed a gradation which corresponded roughly with the gradation in the fat-soluble organic factor content of the diet (see Table II). The non-irradiated rats on diet *AA* grew even slightly better than the irradiated rats on the same diet. There were two males in the former group and only one in the latter. That easily accounts for the slight difference, because males on a normal diet always grow better than females. But on the deficient diets *B*, *C*, *D*, *E* and *F* the non-irradiated rats did not grow nearly as well as the irradiated ones on the corresponding diets.

¹ The mothers of these litters were kept during pregnancy and lactation on a diet consisting of white bread, oats, maize, bran, cabbage leaf and winter milk. This diet is not very rich in fat-soluble organic factor.

Table II. *Non-irradiated Control Rats.*

No. of rat	No. of litter	Sex	Diet	Initial age days	Final age days	Days on diet	Initial weight grams	Final weight grams	Gain in weight grams	Average amount of food eaten daily grams	In bones			Histological diagnosis
											% H ₂ O	% Ca wet	% Ca dry	
G 596	xxiii	♀	A	22	78	56	29	130	101	11.6	42.35	11.67	20.81	Normal
G 597	xxiv	♂	A	22	78	56	19	137	118	11.6	45.93	11.28	20.86	
G 598	xxv	♂	A	21	77	56	30	165	135	12.4	41.60	11.54	19.65	
G 599	xxvi	♀	A	20	76	56	23	149	126	12.4	42.18	12.59	21.78	
				Averages	25	145	120	12.0	43.01	11.77	20.77			
G 604	xxiii	♀	B	22	78	56	27	89	62	11.5	50.06	8.43	16.87	Moderate rickets and slight osteoporosis
G 605	xxiv	♂	B	22	78	56	29	126	97	11.5	46.24	9.96	18.53	Slight rickets and slight osteoporosis
G 606	xxv	♂	B	21	77	56	36	116	80	12.8	47.00	9.35	17.63	Very slight osteomalacia
G 607	xxvi	♀	B	20	76	56	30	116	86	12.8	40.52	11.85	19.93	Nearly normal
				Averages	30.5	112	81	12.2	45.95	9.89	18.24			
G 612	xxiii	♀	C	22	78	56	27	76	49	9.1	47.50	8.67	16.50	Very slight osteomalacia
G 613	xxiv	♂	C	22	78	56	28	74	46	9.1	48.83	7.26	14.18	" rickets
G 614	xxv	♂	C	21	77	56	36	93	57	10.1	47.53	9.23	17.53	" "
G 615	xxvi	♀	C	20	76	56	31	88	57	10.1	46.73	8.48	15.93	" "
				Averages	30.5	82	52	9.6	47.64	8.41	16.03			
G 620	xxiii	♀	D	22	78	56	29	83	54	9.7	46.63	7.34	13.73	Slight rickets and slight osteoporosis
G 621	xxiv	♂	D	22	78	56	28	103	75	9.7	47.62	7.67	14.63	Very slight healing rickets and moderate osteoporosis
G 622	xxv	♀	D	21	77	56	34	88	54	9.9	41.62	10.16	17.36	Slight osteomalacia and osteoporosis
G 623	xxvi	♂	D	20	76	56	36	112	76	9.9	48.50	9.25	17.87	Very slight osteomalacia and severe osteoporosis
				Averages	32	96	64	9.8	46.05	8.60	15.89			
G 628	xxiii	♂	E	22	78	56	33	91	58	10.3	48.05	8.28	15.94	Very slight rickets and slight osteoporosis
G 629	xxiv	♀	E	22	78	56	33	68	35	10.3	46.90	7.73	14.57	" "
G 630	xxv	♂	E	21	77	56	30	63	33	8.5	45.51	8.77	16.11	Very slight osteomalacia
G 631	xxvi	♀	E	20	76	56	24	54	30	8.5	49.69	6.94	13.77	Slight rickets
				Averages	30	69	39	9.4	47.53	7.93	15.09			
G 636	xxiii	♀	F	22	78	56	31	68	37	8.0	48.80	8.37	16.36	Slight rickets and moderate osteoporosis
G 637	xxiii	♂	F	22	78	56	36	51	15	8.0	44.85	8.13	14.72	Severe osteoporosis
G 638	xxiv	♂	F	22	78	56	33	72	39	8.0	50.48	6.86	13.88	Moderate rickets and osteoporosis
G 639	xxv	♀	F	21	77	56	41	111	70	9.6	43.40	9.22	16.34	Very slight healing rickets and very slight osteoporosis
G 640	xxvi	♀	F	20	76	56	28	55	27	9.6	45.73	7.50	13.84	Moderate rickets and slight osteoporosis
				Averages	34	71	37	8.64	46.65	8.10	15.02			

The irradiated rats on diets *AA*, *B* and *C* grew equally well and reached normal weights for rats of that age and breeding. The average maximum gain in weight and average final weight of the irradiated rats on diet *D* were only slightly less (7.5 %) than those on diet *AA*. But the irradiated rats on diets *E* and *F*, although they grew very much better than the non-irradiated controls on the corresponding diets, did not grow as well as those on the other four diets. Towards the end of the experimental period the growth of the irradiated rats on diets *E* and *F* had practically stopped, while the irradiated rats on the other diets were still making regular weekly gains in weight (see Table III).

Table III. *Irradiated Rats.*

No. of rat	No. of litter	Sex	Diet	Initial age days	Final age days	Days on diet	Initial weight grams	Final weight grams	Gain in weight grams	Average amount of food eaten daily grams	In bones			Histological Diagnosis
											% H ₂ O	% Ca wet	% Ca dry	
<i>G</i> 600	XXIII	♀	<i>AA</i>	22	78	56	27	129	102	12.2	43.23	12.84	22.64	Normal
<i>G</i> 601	XXIV	♀	<i>AA</i>	22	78	56	22	128	106	12.2	40.37	12.53	21.02	"
<i>G</i> 602	XXV	♂	<i>AA</i>	21	77	56	31	168	137	11.1	43.13	11.77	20.72	"
<i>G</i> 603	XXVI	♀	<i>AA</i>	20	76	56	25	109	84	11.1	46.38	11.37	21.22	"
Averages							26	133.5	107	11.6	43.27	12.12	21.40	
<i>G</i> 608	XXIII	♀	<i>B</i>	22	78	56	29	126	97	12.8	39.58	12.45	20.61	Normal
<i>G</i> 609	XXIV	♂	<i>B</i>	22	78	56	28	139	111	12.8	43.83	11.41	20.32	"
<i>G</i> 610	XXV	♀	<i>B</i>	21	77	56	36	122	86	13.3	39.80	12.50	20.74	"
<i>G</i> 611	XXVI	♂	<i>B</i>	20	76	56	29	160	131	13.3	42.02	11.73	20.29	"
Averages							30.5	137	106	13.0	41.30	12.02	20.49	
<i>G</i> 616	XXIII	♀	<i>C</i>	22	78	56	30	122	92	13.3	37.52	12.82	20.51	Normal
<i>G</i> 617	XXIV	♂	<i>C</i>	22	78	56	29	139	110	13.3	40.74	11.34	19.16	"
<i>G</i> 618	XXV	♀	<i>C</i>	21	77	56	35	150	115	13.0	40.58	10.37	17.46	"
<i>G</i> 619	XXVI	♀	<i>C</i>	20	76	56	31	122	81	13.0	39.53	12.04	19.91	"
Averages							31	133	99	13.1	39.59	12.06	19.86	
<i>G</i> 624	XXIII	♀	<i>D</i>	22	78	56	30	108	78	13.1	39.79	13.12	21.79	Normal
<i>G</i> 625	XXIV	♀	<i>D</i>	22	78	56	31	147	116	13.1	42.20	11.82	20.46	"
<i>G</i> 626	XXV	♀	<i>D</i>	21	77	56	33	105	72	13.6	39.84	11.44	19.02	"
<i>G</i> 627	XXVI	♀	<i>D</i>	20	76	56	34	133	99	13.6	38.14	12.71	20.55	"
Averages							32	123	91	13.3	39.99	12.27	20.45	
<i>G</i> 632	XXIII	♀	<i>E</i>	22	78	56	32	—	—	—	—	—	—	Severe osteoporosis
<i>G</i> 633	XXIV	♂	<i>E</i>	22	78	56	33	—	—	—	—	—	—	Not examined
<i>G</i> 634	XXV	♂	<i>E</i>	21	77	56	28	106	78	12.0	38.84	11.48	18.79	Nearly normal
<i>G</i> 635	XXVI	♀	<i>E</i>	20	76	56	24	108	84	12.0	40.84	11.64	19.70	"
Averages							26	107	81	12.0	39.84	11.56	19.24	
<i>G</i> 641	XXIII	♀	<i>F</i>	22	78	56	31	96	65	15.1	35.55	12.66	19.67	Nearly normal
<i>G</i> 642	XXIV	♂	<i>F</i>	22	78	56	33	128	95	15.1	41.62	11.25	18.69	"
<i>G</i> 643	XXV	♂	<i>F</i>	21	77	56	38	141	103	13.3	40.63	11.26	18.97	"
<i>G</i> 644	XXVI	♀	<i>F</i>	20	76	56	26	105	79	13.3	43.34	11.37	20.06	"
Averages							32	117	85	14.2	40.28	11.63	19.35	

Gross Autopsy Findings.

The irradiated rats on diets *AA*, *B*, *C* and *D* were well developed and nourished and presented no gross abnormalities of their bones. The two rats on diet *E* which died early in the experiment had acute haemorrhagic enterocolitis and their bones were thin and very brittle. The remaining two rats on diet *E* and those on diet *F* differed from those on the other four diets only in

the fact that their bones were slightly thinner and not quite as firm. One rat on diet *F* was found pregnant with eight well-developed embryos. This is interesting, since it is well known that on a — *A* diet without irradiation rats rarely become pregnant.

The non-irradiated rats on diet *AA* were normal in every respect. Grossly the bones resembled those of the irradiated rats on diets *AA*, *B*, *C* and *D*. On diet *B* two of the non-irradiated rats showed very slight enlargement of the costo-chondral junctions and some indistinctness and irregularity of the costo-chondral line. The same occurred in two of the non-irradiated rats on diet *C* and in one of these fractures of several ribs were present. In all the non-irradiated rats on diet *D* the costo-chondral junctions were very slightly enlarged, the costo-chondral lines were not quite distinct, and the costal portion of the ribs was easily bent and broken, but no spontaneous fractures were present. On each of the diets *E* and *F* two rats had numerous spontaneous fractures of the ribs. The costo-chondral junctions were moderately enlarged in all, and the costo-chondral lines in most were very indistinct and irregular. The costal portion of the ribs and the shafts of the long bones were easily bent and broken.

Histology.

In a former communication one of the authors [Goldblatt, 1923] gave a full description with photographic illustrations of the various degrees of bone pathology which occurred in non-irradiated rats on diets similarly graded in their content of fat-soluble organic factor. The same descriptions and illustrations hold true for the diagnoses given in Tables II and III of this communication. But on every deficient diet in the present study the number of rats that developed a degree of rickets or of osteomalacia was greater than in the previous investigation (see Table I). This is undoubtedly attributable to the fact that the litters were very large and that, as was stated in the beginning, the mothers of these litters were kept on a diet not very rich in fat-soluble organic factor during pregnancy and lactation. Of the rats that were irradiated daily, not one developed the slightest sign of rickets or of osteomalacia. Of those that were on diets *AA*, *B*, *C* and *D*, the bones were normal in every respect and could not be distinguished. The bones of the irradiated rats on diets *E* and *F* were nearly normal, differing from the others only in the fact that the cortical bone was slightly thinner and the trabeculae shorter, thinner and fewer, though very well calcified. This tendency for the trabeculae to be thin was noticed in some of the irradiated rats on all of the deficient diets (*B*, *C*, *D*, *E*, and *F*).

Investigation of the Calcium Content of the Bones.

Chemical analysis for calcium content of both femora, tibiae and fibulae of all the rats was made by Aaron's method, as described in a previous communication by Goldblatt [1923]. The detailed results are given in Tables II and III, and the averages in Table IV. They show that in the non-irradiated

rats a gradation occurred in the percentage of calcium deposited in the bones which corresponded roughly with the gradation in the fat-soluble organic factor content of the diets. This is a similar result to that obtained previously [Goldblatt, 1923], but the gradation is not quite as regular. The percentage of calcium in the bones of the non-irradiated rats on diet *AA* was slightly less than that of the irradiated rats on the same diet, but the difference was very slight, being only 2.9 % less per wet and 3 % per dry weight of the bones. In a previous experiment the authors [Goldblatt and Soames, 1922] showed that rats of good stock, if kept on a perfectly normal diet in darkness, grow as well and their bones develop and calcify as well as those kept in a well-lighted room supplemented with irradiations by a mercury-vapour quartz lamp for ten minutes daily. The slight difference noted above is probably due to the fact that the rats used did not have a large pre-experimental store of fat-soluble organic factor. Rats of that kind were used expressly in this investigation in order to give a very severe test to the efficacy of the light rays.

In the bones of the irradiated rats on diets *AA*, *B*, *C* and *D*, little or no difference was found in the average percentage of calcium, it being normal in the four groups for rats of that age and breeding (see Table IV). The bones of the irradiated rats on diets *E* and *F* did not calcify quite as well as the others, though very much better than those of the non-irradiated rats on the corresponding diets.

Table IV.

Diet	In bones of non-irradiated rats			In bones of irradiated rats		
	Average % H ₂ O	Average % Ca wet	Average % Ca dry	Average % H ₂ O	Average % Ca wet	Average % Ca dry
<i>AA</i>	43.01	11.77	20.77	43.27	12.12	21.40
<i>B</i>	45.95	9.89	18.24	41.30	12.02	20.49
<i>C</i>	47.64	8.41	16.03	39.59	12.06	19.86
<i>D</i>	46.05	8.60	15.89	39.99	12.27	20.45
<i>E</i>	47.53	7.93	15.09	39.84	11.56	19.24
<i>F</i>	46.65	8.01	15.02	40.28	11.63	19.35

SUMMARY AND CONCLUSIONS.

The results of macroscopical, microscopical and chemical investigation agree in showing that rats on a diet as deficient in fat-soluble organic factor as even diet *D* of this study, can grow normally and their bones develop and calcify normally provided they are irradiated for about ten minutes daily by a mercury-vapour quartz lamp.

But the light rays cannot act as a substitute for the fat-soluble organic factor. When the latter is completely absent from the diet (diet *F*), irradiation, though it causes rats on that diet to grow much better and their bones to develop and calcify much better than non-irradiated controls, yet does not bring about absolute normality in either respect. The degree to which such rats approach the normal probably depends upon the pre-experimental store of fat-soluble organic factor which the animals possess.

When rats are used which have a poor pre-experimental store of fat-soluble organic factor, if they are not irradiated, grading the content of that factor in their diet results in a corresponding gradation of the percentage of calcium in their bones. Some of the non-irradiated rats on the diets deficient in the organic factor (*B*, *C*, *D*, *E* and *F*) develop slight to moderate degrees of osteomalacia or rickets, complicated in most instances by some degree of osteoporosis. This confirms the results obtained in another investigation by one of the authors [Goldblatt, 1923].

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