CLXXXI. A PRELIMINARY NOTE ON THE GROWTH-PROMOTING AND ANTI-RACHITIC VALUE OF COD LIVER OIL WHEN INJECTED INTRAPERITONEALLY.

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ATTEMPTS have been made by various workers to confer on animals the same protection from a deficiency of the fat-soluble organic factor, by the subcutaneous injections of cod liver oil, as is obtained by its ingestion. Zucker and Matzner [1924] injected the active principle of cod liver oil into rachitic rats and found that no healing was produced; from this they deduced that the active principle must operate through the intestinal tract, thus supporting the theory of Cramer [1923].

That fat can be absorbed when injected either subcutaneously into human beings or intra-tracheally into rabbits has been shown by Schick, Fries, Kohn and Cohen [1924] and by Bossan and Guieysse-Pellissier [1919]; and that the injection of cod liver oil into rats may promote growth, by Wollman and Vagliano [1923]. These authors found cod liver oil and butter fat to be toxic when administered parenterally and only one animal, on a small dosage, survived in the first experiment. The exudate from abscesses was in all cases sterile. I noticed no such effect from the injection of sterile cod liver oil. Wollman and Vagliano conclude from their experiments that fat-soluble A will produce the same effect whether administered by mouth or parenterally, though in the latter case growth is retarded by irregularity of absorption.

Blegvad [1924] injected the active principle of cod liver oil into a patient suffering from night-blindness and observed marked improvement after a fortnight's treatment.

The results of these workers are conflicting and in order to obtain a definite answer to the question as to whether the action of cod liver oil necessarily takes place through the intestinal tract or no, a preliminary experiment on intraperitoneal injections has been carried out.

EXPERIMENTAL.

One series of animals, divided into three groups, was put on McCollum's Diet 3143 [1921]. This is a severe rickets-producing diet.

Group I received Diet 3143 + 0.25 cc. sterile cod liver oil by mouth.

„	II	„	„	„	„	„	intraperitoneally.
„	III	„	„	without oil,	to serve as controls.		

The experiment lasted 21–29 days with different litters, and the administration of the cod liver oil was carried out twice weekly during that time. Great care was taken that there should be no oozing of the oil after the injections and the skin was cleaned with light petroleum to remove any trace of oil left by the hypodermic needle. In any case where oozing of oil from the dermic puncture, after severe massage, occurred, the animal was discarded. The oil was sterilised in the autoclave for 1 hour at 120°; none of the animals developed either abscesses or peritonitis.

Post-mortem Findings.

Group I. The rib bones were in all cases firm. The costo-chondral junctions appeared normal except in two cases where a slight cupping was seen.

Group II. Large quantities of non-absorbed oil were found as bright yellow, opaque, beads thickly studding the omentum and, to a lesser degree, the mesentery, peritoneum and fat round the kidneys. The largest masses were hard and firmly adherent to the omentum. These, on microscopic examination, were found to consist of conglomerations of small globules of cod liver oil surrounded by fibrous tissue. The rib bones were somewhat soft and in one or two cases fractures were found and slight angulation at the costo-chondral junctions seen. The general picture in this group was that of moderate rickets.

Group III. All the rats of this group presented an appearance of severe rickets. The bones were soft and in most cases there were multiple fractures of the ribs with angulation at the costo-chondral junctions. Table I gives the histological findings for this series. From this table it will be seen that the

Table I.

No. and sex of rat	Litter	Days on exp.	Diet	Post-mortem findings	Histology	Remarks
Group I						
133 ♀	A	21	3143	Normal	Normal	
134 ♀	A	21	+	"	"	
153 ♂	B	29	0.25 cc.	"	"	
154 ♂	B	29	oil by mouth	"	"	
155 ♂	B	29	mouth	"	"	
169 ♀	C	26	"	"	"	
170 ♂	C	26	"	"	"	
Group II						
131	A	21	3143	Rickets +	Rickets + +	No fractures or angulation
132	A	21	+	" +	" + + +	" "
152 ♂	B	29	0.25 cc.	" + +	" + + + +	Multiple fractures and slight angulation
174 ♀	C	26	oil	" +	" + + +	" "
175 ♀	C	26	injected	" + +	" + + +	Angulation, no fractures
Group III						
135 ♀	A	21	3143	Rickets + + + +	Rickets + + + +	Multiple fractures and angulation
136 ♀	A	21	"	" + + ±	" + + + +	" "
156 ♀	B	29	"	" + + +	" + +	" "
157 ♂	B	29	"	" + + ±	" + + + ±	Angulation, no fractures
158 ♂	B	29	"	" + + +	" + + + ±	Fractures and angulation
171 ♀	C	26	"	" + + ±	" + + + ±	Fractures
172 ♀	C	26	"	" + + +	" + + + +	Multiple fractures and angulation
173 ♂	C	26	"	" + + ±	" + + + ±	Fractures and angulation

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protection afforded by intraperitoneal injections of cod liver oil into rats on this diet is slight, but the diet has a very low P and high Ca percentage, and is therefore a drastic one, so that the test is severe and any degree of protection is significant.

A second series of animals was put on a caseinogen diet (F diet), with the following composition:

Inactivated caseinogen	...	...	...	...	...	...	20 g.
Wheat starch	...	...	...	...	...	...	50 „
Cotton-seed oil (hardened)	...	...	...	...	...	...	15 „
Marmite	...	...	...	...	...	...	5 „
Lemon juice	...	...	...	...	...	...	5 cc.
Salt mixture (modified McCollum and Davis, No. 185)	...	...	...	...	...	...	5 g.
Distilled water	...	...	...	...	...	...	50 „

This diet is deficient in the fat-soluble organic factor. The animals were divided into the same three groups as in Series I and were treated in the same manner. Chart I shows the comparative growth of animals in each group. Each curve represents the average growth for all animals of one litter in that group.

After death, the hind legs (femur, tibia, and fibula) were analysed for calcium content by the method of Aron and Sebauer [1910]. The results are given in Table II.

Table II.

Litter, no. and sex of rat	Diet	Initial age (days)	Final age	Days on diet	Bone analysis			Histology	
					Water %	Ca % wet	Ca % dry		
Group I									
D 115 ♀	F +	28	85	57	37.59	12.78	20.47	Normal	
D 116 ♀	0.25 cc.	28	85	57	38.96	11.76	19.26		
D 117 ♂	oil by	28	85	57	37.33	12.72	20.30	"	
E 142 ♂	mouth	29	85	56	38.88	13.35	21.85	"	
E 143 ♂	"	29	85	56	42.80	13.43	23.48	"	
E 144 ♀	"	29	85	56	40.80	12.68	21.43	"	
F 159 ♂	"	28	87	59	39.76	12.30	20.48	Osteoporosis +	
F 160 ♀	"	28	87	59	38.52	12.51	20.35	Normal	
					Av.	39.33	12.69	20.95	
Group II									
D 114 ♀	F +	28	85	57	41.41	11.73	19.52	Normal	
E 137 ♂	0.25 cc.	29	85	56	43.63	11.95	21.20	Nearly normal	
E 139 ♂	oil in-	29	85	56	43.36	10.58	18.67	Osteomalacia ±	
E 140 ♂	jected	29	85	56	43.34	10.94	19.31	Rickets +	
E 141 ♀	"	29	85	56	41.89	12.66	21.75	Normal	
E 148 ♂	"	29	85	56	41.06	11.69	19.84	Nearly normal	
F 165 ♂	"	28	87	59	37.90	11.75	18.92	Normal	
F 167 ♀	"	28	87	59	39.61	11.47	19.00	Osteoporosis ±	
					Av.	41.40	11.60	19.77	
Group III									
D 118 ♀	F	28	85	57	46.67	8.32	15.59	Rickets + ±	
D 119 ♀	"	28	85	57	48.07	8.11	15.63	" + +	
D 120 ♀	"	28	85	57	43.53	7.82	13.84	" +	
E 145 ♂	"	29	85	56	43.22	10.43	18.37	Normal	
E 146 ♀	"	29	85	56	45.17	8.73	15.90	Rickets +	
E 147 ♂	"	29	85	56	45.92	10.26	18.96	" +	
F 162 ♂	"	28	87	59	45.63	8.61	16.04	" + ±	
F 163 ♀	"	28	87	59	Lost	Lost	Lost	Osteomalacia ±	
					Av.	45.46	8.89	16.33	

DISCUSSION.

It will be seen that both the growth and calcification of the animals of Group II are distinctly better than those of the controls of Group III, though

CHART I.

Group I. F diet + 0.25 c.c.
cod liver oil by mouth.

A= Nos. 142 ♂
143 ♂
144 ♀
B= 159 ♂
160 ♀
C= 115 ♀
116 ♂
117 ♂

Group II. F diet + 0.25 cc.
cod liver oil injections.

D= Nos. 137 ♂
139 ♂
140 ♂
141 ♀
148 ♂
E= 165 ♂
167 ♀
F= 114 ♀

Group III.
F diet (- A).

G= Nos. 145 ♂
146 ♀
147 ♂
H= 162 ♂
163 ♀
I= 118 ♀
119 ♀
120 ♀

the values obtained are not equal to those where the oil was administered by mouth.

Intraperitoneal injections were adopted after intramuscular and subcutaneous injections had been found to be unsatisfactory from the point of

view of escape of oil and its possible ingestion by the animal. The injections were limited to two a week. The bulk of the oil was not absorbed, as shown by post-mortem examination, and it is possible that smaller doses administered more frequently would have been better absorbed and thus induced normal growth and calcification.

SUMMARY AND CONCLUSIONS.

Cod liver oil was injected into rats on:

- (1) A rickets-producing diet.
- (2) A diet deficient in the fat-soluble organic factor.

Partial protection was afforded against a rickets-producing diet and improved growth and calcification obtained on a diet deficient in the fat-soluble organic factor, thus demonstrating that the site of action of cod liver oil is not limited to the intestinal tract.

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