CLXIX. BERYLLIUM RICKETS.
II. THE PREVENTION AND CURE OF BERYLLIUM RICKETS.

By HERBERT DAVENPORT KAY and DOROTHY IRENE SKILL.

From the Department of Biochemistry, University of Toronto, and the National Institute for Research in Dairying, University of Reading.

(*Received May 28th, 1934.*)

It was found in 1931 [Branion *et al.*, 1931] that severe rickets could be produced in rats by substituting beryllium carbonate for calcium carbonate in the Steenbock rachitogenic diet. Shortly afterwards the further observation was made that even on the normal stock diet [Bills *et al.*, 1931] of our rat colony with or without extra vitamin D in the form of irradiated ergosterol or cod-liver oil, very severe rickets (demonstrated by X-ray photographs, by the histological appearance of sections of the long bones, by bone ash and plasma-phosphate analyses) might be brought about in growing rats by the simple expedient of adding 0·5 % of beryllium carbonate to this normal diet [Guyatt *et al.*, 1933]. With smaller quantities of the carbonate down to about 0·1 % a milder rickets was produced, amenable in some degree to therapy with the usual antirachitic agents. The addition of an equivalent amount of beryllium phosphate to the Bills diet had no rachitogenic effect—the rats continued to grow normally.

It was shown further that in rats with beryllium rickets, both the inorganic phosphate content of the blood-plasma and the phosphoric ester content of the red blood cell were markedly reduced. This was also shown to be true in other types of experimental rickets [Kay, 1932].

In view of the unusual chemical property of beryllium in forming a phosphate which is relatively insoluble even in fairly acid solutions (p_H 4 or less), *i.e.* at all acidities which are likely to occur in the intestine, it seemed not unlikely that the rachitogenic effect was bound up with this property. The beryllium going into the solution in the acid gastric juice would precipitate quantitatively in the intestine with any free phosphate found there, whether derived from the food or contained in secretions entering the intestine from various glands. The very insoluble precipitate would be excreted in the faeces and cause a constant drain on the animal's intake from the digested food, or re-intake from the intestinal secretions, of phosphate. It was further suggested [Kay and Guyatt, 1933] that the findings in beryllium rickets, taken together with those of other workers on experimental and human rickets, provided strong evidence for the view that experimental rickets, at least, is primarily a phosphate deficiency disease, and that the effect of vitamin D in physiological quantities is essentially to increase the uptake of phosphate through the gut wall, a view which, though not new, had not been supported hitherto by much weight of evidence. While it was obviously idle to endeavour to dissociate the metabolism of phosphorus from that of calcium, it was considered that variations from one individual to another in the functional efficiency of the gut wall in respect to phosphate uptake might

condition the rather irregular onset of rickets in different individuals of the same species on the same diet and might explain in some measure the marked difference in susceptibility to rickets from one species to another on the same diet.

The experiments recorded in this paper were designed to find out:

A. Whether on an otherwise normal diet, rendered rachitogenic by its beryllium carbonate content, the disease can be prevented by parenteral administration of phosphate.

B. Whether, and with what characteristic changes in the tissues, beryllium rickets can be cured by restoring the rachitic animal to a normal diet, *i.e.* by taking out the beryllium carbonate from the rachitogenic diet.

Experimental.

Albino rats were used, the offspring of mothers living on the Bills stock diet or a slight modification of it. The rats after weaning were divided into groups of eight. They were placed on the experimental diets at 22–24 days of age and kept on them for 21 or 22 days. They were then killed by bleeding after mild anaesthesia, samples of tissues quickly removed and pooled for analysis, one complete hind leg was reserved for X-ray examination, and the bones of the other hind leg were used for determination of the percentage of ash in the dry, fat-free bone.

On a normal diet to which 0·5 % of basic beryllium carbonate had been added, very typical rickets developed rapidly and was fully established in 15–18 days, with all the characteristics previously described [Guyatt *et al.*, 1933].

A. *The prevention of beryllium rickets by the parenteral injection of phosphate.*

One experiment may be described in some detail. Nine groups of 8 animals, with litters well shuffled, were used. To one of these groups (A) the normal diet was fed. To the other eight groups was fed the normal diet to which 0·5 % of beryllium carbonate had been added. Of these eight groups, one group (B) received no other treatment and was found with perfectly typical rickets at the end of 21 days. The next group (C) received in addition 1 ml. daily of 25 % neutral sodium β-glycerophosphate, given subcutaneously in two 0·5 ml. doses. Group D consisted of controls receiving instead of glycerophosphate, 1 ml. daily of a 10 % sodium chloride solution (of approximately the same osmotic pressure as the glycerophosphate), also in two 0·5 ml. doses. The other groups were given diminishing quantities of glycerophosphate or sodium chloride as shown in Table I. Sodium glycerophosphate was given in preference to sodium phosphate, since it is more soluble in water at p_H 7·4 than the latter salt, so that a larger dose can be given in a smaller volume. It is, of course, readily hydrolysed in the body to sodium phosphate.

In Table II are shown values for plasma-inorganic phosphate, plasma- and kidney-phosphatase, phosphoric ester content of red cells and liver, and portion of ester-phosphorus of red cells readily hydrolysable by bone-phosphatase.

From Table II it will be observed that in all the animals with 0·5 % beryllium carbonate in the diet, irrespective of whether they received or did not receive parenteral glycerophosphate, the shortage of available P in their bodies, as demonstrated by the low inorganic P of their plasma and the low ester-P of the red cells and liver, was acute. Nevertheless two groups, C and E, had either normal or almost normal bone formation. It is evident that the parenterally injected and quantitatively far from adequate amounts of sodium glycerophosphate were made use of preferentially by the bone, the other tissues, which

Table I. *Effect of subcutaneous injection of sodium β-glycerophosphate in preventing "beryllium rickets."*

Group	No. of animals in group	Av. gain in wt. over 21-day period g.	Diet and treatment	Results of X-ray examination after 21 or 22 days
A	8	70	Bills's stock diet	Normal. Excellent skeletal development
B	8	24	Bills's stock diet + 0·5 % beryllium carbonate	Typical severe rickets
C	8	39	Diet as for B, but 1 ml. of 25 % Na glycerophosphate at p_H 7·4 given subcutaneously each day	3 animals show very slight rachitic changes. Rest normal though less well grown than the A group
D	8	26	Diet as for B, but 1 ml. 10 % NaCl given subcutaneously each day	Rickets indistinguishable from B group
E	8	32	Diet as for B, but 1 ml. of 10 % Na glycerophosphate given subcutaneously each day	Less free from rachitic changes than group C, but all still nearly normal
F	7	23	Diet as for B, but 1 ml. 1 % NaCl given subcutaneously each day	Severe rickets
G	8	24	Diet as for B, but 1 ml. 5 % Na glycerophosphate given subcutaneously each day	All animals rachitic but much less affected than B or D groups
H	8	31	Diet as for B, but 1 ml. of 2·5 % Na glycerophosphate given subcutaneously each day	Typical rickets, but definitely less severe than in B, D, or I groups
I	8	27	Diet as for B, but 1 ml. 0·5 % Na glycerophosphate given subcutaneously each day	Rickets indistinguishable from B or D groups

Table II.

Group	Inorganic P in 100 ml. of blood (mg.)	Plasma-phosphatase* (units per ml.)	Ester-P in 100 ml. of red blood cells: Total (mg. P)	Ester-P in 100 ml. of red blood cells: Easily hydrolysed (mg. P)	Ester-P in 100 g. liver (mg. P)	Kidney-phosphatase* (units per 100 g.)
A. Normals	7·7	0·96	80	18	92	28
B. Severe rickets	3·2	0·44	35	11	29	16
C. Practically normal	4·0	0·46	59	15	41	17
D. Severe rickets	3·2	0·39	54	10	30	15
E. Almost normal	3·4	0·45	62	14	31	17
F. Severe rickets	3·4	0·33	60	11	22	14
G. Medium rickets	3·6	0·52	64	11	26	17
H. Rickets	3·5	0·53	57	17	28	17
I. Severe rickets	3·7	0·59	52	17	31	18

Groups as in Table I.

* The arbitrary unit for phosphatase in plasma is not the same as the unit of phosphatase in kidney.

grew at a much slower rate than in group A, being still starved of phosphorus. This again would indicate that the production of rickets in the rat is more a question of keeping phosphate out of the body than of any damage to the intimate mechanism of bone deposition. The same remarkable fall in the phosphoric ester content of the red cells and liver previously noticed [Kay, 1932] in rickets

produced in rats by the Steenbock or McCollum rachitogenic diet is again shown. There is also a fall, the significance of which is at present unknown, in the kidney-phosphatase in all the animals receiving beryllium in their diet. It is perhaps to be mentioned here that the inorganic P content of the urine is very much diminished on the beryllium diet, though this has not yet been followed quantitatively.

B. *The rate of "cure" of beryllium rickets.*

The rate of "cure" of beryllium rickets after restoration of the rachitic animal to a normal diet (*i.e.* by taking $BeCO_3$ out again from the otherwise normal diet which had been rendered rachitogenic by the inclusion of 0·5 % $BeCO_3$) was followed by taking X-ray photographs, determining weight changes, the level of inorganic phosphate in plasma, and phosphoric esters in both red cells and liver at intervals after removing the $BeCO_3$ from the diet.

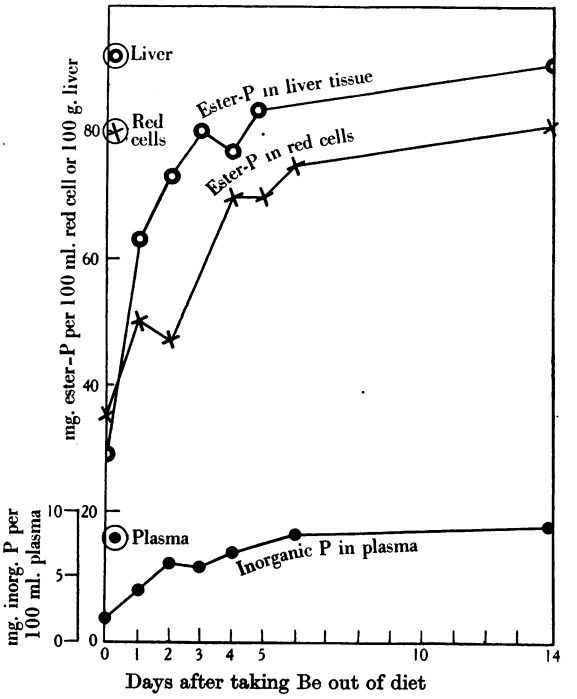

Fig. 1. The isolated points shown on left of graph, each surrounded by a circle, are average figures for the normal content of phosphoric esters in liver, P in red cells and of inorganic P in blood-plasma respectively, of normal animals of an age equal to that of the experimental animals on the day that Be was taken out of the diet of the latter groups.

An experiment of this kind with determinations at intervals of 0, 1, 2, 3, 4, 5 and 14 days after removing the rachitogenic $BeCO_3$ from the diet furnished the data shown graphically in Fig. 1. Other data from the same experiment are given in Table III.

Another experiment on the same lines gave essentially the same results.

It is quite clear that the rate of recovery from beryllium rickets is very rapid. Even after 2 days there are definite signs of healing, and at the end of 3 days, the

Table III. *Recovery from beryllium rickets.*

Except for group A, which received a normal diet from weaning, all the other groups at the age of 23 ± 2 days were given the diet containing 0·5 % beryllium carbonate (but otherwise normal) for another 22 days, then the beryllium carbonate was omitted, leaving the diet strictly normal, and groups of animals were then killed and examined at intervals.

Diet	X-ray findings	Plasma-inorganic P mg. P per 100 ml.	Red cell ester-P mg. P per 100 ml.	Liver ester-P mg. P per 100 g.	Units phosphatase in kidney
Strictly normal stock diet from age of weaning	Normal	7·7	80	92	28
0·5 % Be diet	Rickets + + + +	3·2	35	29	16
Be diet stopped 1 day	Rickets + + + +	4·7	52	63	18
Be diet stopped 2 days	Rickets + + + Definite signs of healing	5·6	46	73	22
Be diet stopped 3 days	Healing rickets	5·3	70	80	28
Be diet stopped 4 days	Healing rickets	5·9	70	78	26
Be diet stopped 5 days	Healing rickets	7·3	75	84	28
Be diet stopped 14 days	Healed rickets	8·2	81	98	25

animal is, radiographically and chemically, well on the way to recovery. It would appear therefore that no very deep-seated, irreversible condition is brought about even in young and rapidly-growing rats by the beryllium feeding. This finding would further strengthen the view that the rachitogenic effect of the beryllium is not the result of possible cell damage due to active toxicity of the, at most, very minute quantity of Be that it is possible to imagine might be taken up from the food without being chemically detectable[1] in the tissues. (Against the toxicity hypothesis must also be ranged the complete inability of Be phosphate to produce rickets when added to the diet.) Nevertheless it must not be imagined that the animals are completely unscathed after the severe disorganisation which has taken place during the period of Be feeding. Even 4 weeks after replacing the rats on the normal stock diet, the X-ray photographs show, at some distance from the now quite normal metaphysis, definite callus-like bands in the long bones in the region which had been occupied by the (hypertrophied) zone of provisional calcification during the period on the Be diet.

Is "beryllium rickets" due to an impurity in the beryllium carbonate?

The suggestion was made to us that some of the effects of the Be diet might possibly be due to fluoride in the basic beryllium carbonate, which is said to contain, occasionally, a little BeF_2. Although this suggestion arose too late to test some of the earlier samples of beryllium carbonate used, two samples derived from the same firm from whom the earlier samples were obtained and a commercial sample from another source contained no detectable quantities of fluoride by the ordinary chemical tests. Moreover, Lamb *et al.* [1933] have recently shown that 0·043 % of sodium fluoride in the otherwise normal diet produces no serious effect in the normal rate of growth of the rat. If our results were even partly due to the toxic effects of fluorine, a large proportion (5 % or more) of the beryllium salt used must have been fluoride, BeF_2.[2] This was certainly not the case in the samples of beryllium carbonate tested. Moreover,

[1] No Be could be found in the ashed bones or ashed tissues (after removing the alimentary canal) of rats fed for three weeks on such a diet, using two qualitative methods [*vide* Guyatt *et al.*, 1933].

[2] Assuming the same toxicity for $\frac{1}{2}BeF_2$ as for NaF, this is probably a safe assumption. $\frac{1}{2}CaF_2$ is much less toxic than NaF (same authors).

when the carbonate was converted into phosphate, by the simple expedient of adding the necessary amount of dilute phosphoric acid and gently evaporating, all the rachitogenic effect disappeared. Also, as just described, even when beryllium is fed, the parenteral injection of sufficient Na glycerophosphate will prevent the rachitic symptoms. Taken together, the evidence appears to be convincing that fluoride poisoning is not concerned in the phenomena of "beryllium rickets."

SUMMARY.

1. Beryllium rickets which invariably results when young rats are given the normal stock diet to which 0·5 % of beryllium carbonate has been added, can be prevented if at the same time that the beryllium is being fed, a relatively small daily quantity of sodium glycerophosphate is administered parenterally. This adds further support to the view that beryllium rickets is mainly or possibly entirely due to defect of absorption of phosphate from the gut.

2. If animals with beryllium rickets are put on to a normal diet without beryllium, they recover very rapidly. One of the first changes to be observed is an increase in the phosphoric ester content of the erythrocytes and of the liver.

The authors wish to express their indebtedness to Prof. H. Wasteneys for his kindness in affording facilities for the completion of this work and for his generous help in other ways both before and after the senior author had left Toronto.

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