SOME OBSERVATIONS ON CALCIUM METABOLISM IN RATS INJECTED WITH CALCIUM TRIPHOSPHATE SUSPENSIONS

by

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In view of the possible use of radioactive phosphorus as a source of radiation injected into tumours in the form of insoluble calcium triphosphate, some experiments were carried out on the time during which ordinary calcium triphosphate is retained in healthy rat muscle. This investigation led to unexpected results, and as the work, though by no means finished, must be interrupted for several months, it seems worth while to publish them. The amount of injected calcium appeared not to diminish regularly from the beginning, but it showed first a distinct increase and it was only later that a diminution set in.

I have carried out only calcium determinations and am aware that these should be supplemented by phosphorus determinations.

0.5 ml of an aqueous suspension of freshly prepared $Ca_3(PO_4)_2$, corresponding to about 20 mg Ca, was injected intramuscularly into the right hind leg of rats. The animals were killed after 1, 4, 8 and 22 days respectively and the amount of Ca in the muscle was determined. The amount of Ca in the normal, non-injected muscle was substracted from the amounts found in the injected rats. Table I shows that the quantity of Ca gradually increased from the 1st to the 8th day after the injection, while after 22 days it had decidedly decreased again.

We do not know the cause of the described phenomena. It is certainly not impossible that the increase of Ca has some relation to the inflammatory process, which always developed as a consequence of the Ca phosphate injection. This point too requires further investigation.

EXPERIMENTAL PART

The calcium triphosphate* was prepared by mixing 40 ml of an aqueous solution containing 8.48 g K_3PO_4 and 30 ml of an aqueous solution containing 6.66 g $CaCl_2$. The precipitate formed was separated by centrifuging and washed three times in the centrifuge, using 50 ml of water each time. After pouring off the last washing fluid, 5 ml water was added to the precipitate. The suspension obtained in this way was used for the injection.

Rats (body weights varying from 220 to 280 g) were injected with 0.5 ml of the suspension into the m. gastrocnemius of the right hind leg. A 1 ml syringe was used. The suspension was freshly prepared and always thoroughly mixed before drawing it into the syringe. No sterilisation was used. The animals did not seem to suffer from the injection with the passage of time.

After decapitation and removal of the skin, the muscles of each right hind leg together with the fluid mass (see Table I) were transferred to a Pt crucible and incinerated with a few drops of H_2SO_4 and HNO_3. The ash was dissolved in HCl and the Ca precipitated with NH_4-oxalate at slightly acid reaction. The oxalate precipitated was determined by titration with K permanganate according to

* According to CH. HIGGINS[1] $Ca_3(PO_4)_2$ in aqueous suspension is converted into hydroxyapatite

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the procedure described by KNIPHORST[2], by which method disturbances, caused by the presence of other metals and phosphate are avoided. It is of particular importance to follow this procedure exactly in determinations of the Ca in the muscles of non-injected rats. 1 g of these muscles contains about 0.1 mg Ca. The average weight of the muscles incinerated amounted to 8 g; hence 0.8 mg Ca had to be substracted from the results of the analysis of the injected muscles in order to compare them with the injected quantity of Ca. The values given in Table I were obtained in this way.

After many preliminary experiments which all gave the same general result (even when 40 mg Ca was injected into one rat), the final experiment, the results of which are collected in Table I, was carried out. 3 rats were killed after 1·24 hours, 5 rats after 4·24 hours, another 5 rats after 8·24 hours, and 3 rats after 22·24 hours.

TABLE I

RATS INJECTED WITH AN AMOUNT OF $Ca_3(PO_4)_2$ CORRESPONDING WITH 20 mg Ca

Rat No.	Time after injection	Residual amount of Ca (mg)	Remarks
1	1·24 hours	20.9	The suspension was distinctly circumscribed in (or between) the muscles
2		22.0	
3		21.8	
4	4·24 hours	24.2	Some exudate could be seen in (or between) the muscles at the site of the injection
5		27.7	
6		26.9	
7		24.0	
8		27.6	
9	8·24 hours	29.7	Much fluid, containing many leucocytes
10		31.1	
11		26.3	
12		30.3	
13		31.1	
14	22·24 hours	9.4	No fluid remaining, and the deposited Ca phosphate had a granular appearance
15		12.3	
16		20.1	

The accuracy of injecting a certain amount of Ca was checked in the following way: after 4 rats had been injected, one sample was taken with the same syringe and the Ca determined immediately. The results of these determinations were 20.3, 19.8, 19.9 and 20.5 mg Ca, which shows the constancy of the amounts injected.

SUMMARY

The amount of calcium, deposited in the muscle after injection of Ca in the form of $Ca_3(PO_4)_2$ suspensions into the leg muscles of rats, increases in the course of the first 8 days after the injection. After 22 days it has definitely decreased again to amounts equal to or below the amount injected.

RÉSUMÉ

La quantité de calcium déposée dans le muscle après l'injection de calcium sous forme de suspension de $Ca_3(PO_4)_2$ dans les muscles des pattes de rats croît au cours des premiers 8 jours après l'injection. Après 22 jours, cette quantité a nettement baissé, et devient inférieure ou au plus égale à la quantité injectée.

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ZUSAMMENFASSUNG

Die Menge Calcium, die nach Calciuminjectionen in Form von $Ca_3(PO_4)_2$-Suspensionen in die Beinmuskeln von Ratten in diesen Muskeln abgesetzt wurde, nimmt im Laufe der ersten 8 Tage nach der Injektion zu. Nach 22 Tagen hat sie wieder entschieden abgenommen zu Mengen, die der injizierten gleich sind oder weniger betragen.

REFERENCES

[1] CH. HIGGINS, *Physiol. Rev.*, 17 (1937) 122.
[2] L. C. E. KNIPHORST, *Chem. Weekblad*, 43 (1947) 54, 66.

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