B.F.GOODRICH CHEMICAL COMPANY
Inter-Organization Correspondence

To: John Nelson | Date: May 21, 1969

Location: Cleveland | From: L.B. Crider

Subject: Review of Hand Problem

COMPANY CONFIDENTIAL

Prior to our meeting on May 29th to discuss the status of this problem, I thought it would be well to set down on paper some of our current thinking about the results obtained since our last review. I would also like to make some comments about the direction in which our future research relating to this problem should be headed.

With respect to our findings since our last review, I have enclosed a summary of Dr. Eirichs objectives and conclusions including the results from both his 1968 and 1969 reports. Hopefully, this will relieve you of some of the agony of sorting out these facts from the rather lengthy, narrative description of his work. There are several significant facts that have been born out of this work. Firstly, is his discovery that slight imbalances in calcium and phosphate ions have a substantial effect on the soluability of hydroxyapatite (HAP). This finding is particularly significant when one considered the possible anesthetic action of VCl monomer on the osteoblast and osteoclast cells.

Another significant result from Dr. Eirichs' work (although a major portion of this phase was done at the Development Center) is that it has been established that some small amount (500-1000ppm) of VCl is bound to collagen. Eirich's postulation that this occurs at chain ends is a reasonable rationalization but remains to be established. The method that he has developed for measuring decalcification and recalcification of bone tissue can be used by Dr. Motzkin in her studies involving living bone tissue. Dr. Eirich's theory regarding the effects of VCl monomer on the function of the lipid portions of living cells can perhaps be explored by this method.

There has also been a considerable advancement in our over all knowledge of the metabolism of bone tissue from the various articles and reviews published in the literature during the past year. Most significant of these is the work showing that the initial growth of HAP occurs in "holes" in the collagen fibrils. A number of agents are effective in blocking recalcification of bone tissue by reacting with the Σ-NH_2 groups in collagen. A theory has been proposed that the hydrophobic character of the blocking agents is effective in excluding water from the "holes" in the fibrils thus preventing the initial growth of HAP.

Perhaps even more important are the new analytical methods that are suggested for measuring decalcification, in vivo, by monitoring the level of hydroxyproline in urine. It has now been established that major disruptions in bone metabolism can be related to gross changes (increase) in the hydroxy proline level in the urine. This applies to tests in a wide variety of test animals as well as humans. I have also included in my attached [illegible] of some of this data taken from the literature.

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In our meeting on May 29, I think it would be well if we could also arrive at some decisions regarding the type of proof required for a conclusive solution to this problem. One of the things that I have been concerned about is that we can go on ad infinitum in isolating components that can have an effect on the lysing of bone tissue but unless we can see this effect in the x-rays of test animals we will never be convinced that we have found a cause for the problem. The MCA results clearly show that only a small percentage of the total population exposed are vulnerable to this problem. If we can expect this same frequency in test animals the expectations of producing a positive result detectable by x-ray are quite remote.

It is my feeling that a more sensitive indicator is needed. If we could, for example, show that exposure to various agents (VCl monomer, VCl peroxide, poly scraping, etc.) causes increases in hydroxyproline in the urine or increased calcium or phosphate levels in solid excrement we would then have a positive indicator that we have isolated the material that effects bone matabolism. The results of this type of testing would also be available in a much shorter period of time. As a minimum it should be incorporated in our present test program at Kettering. Data relating to the use of these test methods in problems involving the resorption of bone tissue are now available. We also have in hand the analytical test methods for this type of study.

L.B. Crider

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Summary of Dr. Eirich's work contained in 1968 report:

Objectives

1. Determine the absorption of gelatin on hydroxy apatite (HAP)
2. Measure binding of calcium and phosphate ions on gelatin.
3. Study the effects of gelatin on the precipitation of HAP.
4. Measure the binding of VCl monomer on gelatin.

Conclusions

1. Gelatin is strongly absorbed on HAP. An absorption isotherm of gelatin on HAP was obtained. In the presence of VCl monomer the initial absorption of gelatin on HAP is suppressed.
2. The binding of calcium and phosphate ions to gelatin is small and not much dependent on pH. Ion binding is not effected by VCl monomer.
3. The precipitation of HAP is only slightly affected by VCl monomer.
4. The degree of binding of VCl monomer on gelatin is so slight that it cannot be measured

Summary of Dr. Eirich's work contained in 1969 report:

Objectives

1. Study the effects of VCl monomer on the precipitation of hydroxyapatite.
2. Continue work on measuring the direct binding of VCl on collagen or gelatin.
3. Study changes in collagen structure induced by VCl monomer.
4. Develope a method for measuring the rate of calcification and decalcification of bone tissue.
5. Complete basic study of decalcification of bone slices.
6. Study the affects of VCl monomer on decalcification rate.

Conclusions

1. Radioactive tracer methods were used to study the precipitation of HAP in vitro. The results obtained are at considerable variance with the data in the literature. It was noted that potassium ions in particular have a sustantial effect on the solubility of HAP. This finding is of utmost importance since it points to the possibility that minor biochemical imbalances of sodium and potassium could be caused by the action of VCl monomer on the lipid portion of cells.

2. The amount of VCl bound to collagen is of the order of 500-1000 ppm. This is approximately one VCl per collagen chain end group. The binding of VCl is stronger at neutral than at acid pH which also fits the picture of end-group binding.

3. VCl monomer does not cause any disordering of collagen structure that can be observed by electron microscope.

4. A method was developed for measuring the rate of calcification and decalcification of bone tissue using sedimentation methods.

5. Studies in vitro show two distinct regimes of decalcification, a slower rate followed by one that is faster. The initial slow rate is thought to be diffusion controlled.

6. Although it is too early to arrive at conclusive observations, the presence of VCl monomer definitely seems to change the decalcification process.

Summary of data from the literature

Reconstituted collagen fibrils as well as collagen fibrils from a wide variety of tissues and demineralized collagen from bone are effective nucleation calalysts, and are readily able to induce the formation of apatite crystals from solutions that do not form crystals in the absence of collagen.

The manner in which collagen macromolecules are packed in native fibrils....steric relationships between amino acid groups....charge distribution.. ..space geometry...are factors that facilitate the induction of apatite crystals.

The small apatite crystallites, initially deposited in collagen, in vitro, are located in specific regions of collagen fibrils which we now know to correspond to "holes" in the fibrils.

There is no established relationship between the 700A repeat spacing in collagen fibrils and calcification.

The blocking of Σ-NH_2 groups with fluoro-2, 4-dinitrobenzene in vitro completely blocks calcification of collagen. Blocking is also accomplished with carbobenzoxy chloride. This reaction is reversible.

The above does not establish that Σ-NH_2 groups are themselves involved in nucleation. The bulky dinitrophenyl or carbobenzoxy groups could distort or sterically shield the other adjacent groups that are vital to recalcification.

The recalcification of collagen having modified Σ-NH_2 groups is effected by the exclusion of water by the modifying agents.

The modification of the Σ-NH_2 groups by hydrophobic groups located in the "holes" can effect recalcification by excluding water from the holes.

Methylation of the carboxyl groups in collagen inhibits in vitro recalcification of bone tissue.

There are numerous references in the literature that osteomyelitis and collagen resorption in humans, rats and other test animals is reflected by increased urinary hydroxyproline.

Some interesting work has been reported on the effects of estrogens on collagen matabolism. It is known that collagen in the uterus increases about 8 fold during pregnancy and resorption is complete about 3 weeks after post partum. β-Estradiol has a marked effect on retarding the degradation of collagen during the first 4 days post partum.