UPTAKE OF GOLD BY COLLAGEN IN GOLD THERAPY

BY

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Gold in the form of thio-complexes has been successfully used in the treatment of rheumatoid arthritis for nearly 40 years (Lande, 1927; Pick, 1927), but the mode of action of gold compounds on the diseased connective tissue has yet to be elucidated.

In our experiments we have tried to demonstrate the direct binding of gold by collagen because it may be assumed that this type of reaction is similar to that occurring with other heavy metals as described by other authors (Gustavson, 1956).

As far as the tanning mechanism of gold therapy is concerned, the degree of intra-vital and extra-vital staining has to be taken into account at the same time.

**Material and Methods**

Rats of the Wistar strain (*Rattus norvegicus* var. *alba*) of initial body weight of 100 g. were divided into two groups:

1. Animals in which sodium gold thiosulphate (SGTS)* was administered intramuscularly once a week in doses of 2 mg./100 g. body weight. Staining resulting from the treatment will be referred to as "intra-vital" or *in vivo* staining.
2. Control animals.

Tail tendon collagen fibres were obtained in the usual way. For electron microscopy† collagen fibres from treated rats were cut on a freezing microtome into pieces approximately 3 μ in length. These pieces were suspended in distilled water and quickly mounted on collodion-filmed supporting grids.

For "extra-vital" (*in vitro*) staining collagen fibres were treated with:

1. 1·0 per cent. solution of SGTS;
2. 0·1 or 0·001 M gold trichloride (AuCl₃) aqueous solution with subsequent washing in Britton-Robinson phosphate buffer solution (Delory and King, 1945).

The stability of the collagen structure was determined in Britton-Robinson phosphate buffer solution (pH 2·3; \( \mu = 0·0125 \)) by the following quantitative methods:

1. The shrinkage temperature (\( T_s \)) was measured microscopically according to the method of Borasky and Nutting (1949), with the fibre submerged in the Britton-Robinson buffer solution, at pH's ranging from 2·0 to 10·0 (\( \mu = 0·0125 \)).
2. Swelling was determined in Dogadkin's apparatus as a decrease in the liquid volume;
3. Contraction and relaxation
   a. Under constant load (50 mg.);
   b. Under variable load, for calculating the molar concentration of cross-linkages according to Flory's equation (1953).‡

**Results**

Intra-vital uptake of sodium gold thiosulphate (SGTS) by collagen could not be detected electron microscopically until after 8 weeks' treatment with SGTS. Electron micrographs of specimens from earlier stages of treatment did not differ from those of collagen from untreated control animals. Collagen from rats treated for 8 weeks with SGTS exhibited a distinct structure with four bands in each period, two of which appeared comparatively dark (Fig. 1, opposite).

It must be pointed out that not all collagen fibres stained intra-vital to the same extent. Well-stained fibres and those showing only poor structure occurred in the same specimen.

\[ \frac{f/A}{A} = \nu RT \gamma^2 (\alpha - \alpha^2) \]

When \( f/A \) = the force used and related to the cross section unit of the swollen fibre, \( R \) = gas constant, \( T \) = absolute temperature, \( \alpha \) = relative prolongation of the fibre, \( v \) = effective degree of cross-linkage (moles per cc.), \( v_r \) = volume ratio of the dry polymer in the swollen sample.

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* Sanocrysin-Dansk Chemo-therapeutisk Selskab
† The prototype of the Czechoslovak electron microscope made by the Institute for Instrument Research of the Czechoslovak Academy of Sciences (Brno) was used.

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Fig. 1.—Electron micrograph of rat tail tendon collagen stained intravitally with gold thiosulphate.

(1) Shrinkage Temperature.—An obvious increase in $T_s$ values, especially in alkaline media, was observed in collagen fibres from rats treated intravitally with SGTS (Fig. 2). $T_s$ values increased with the duration of SGTS treatment.

Treatment of native collagen fibres with AuCl$_3$ in vitro also resulted in a distinct increase in $T_s$ values (Fig. 3), particularly in neutral and alkaline media. The maximum $T_s$ values were obtained with 0·1 M AuCl$_3$ treatment at all pH values above 4·0, and with 0·001 M AuCl$_3$ treatment at pH values above 6·0. No changes in $T_s$ were observed in native collagen fibres treated with SGTS in vitro. An increase in $T_s$ (up to 83°C.) was observed after decomposition of SGTS, due to oxidation.

In order to compare the effects of gold compounds in vivo and in vitro, the dependence of the $T_s$ values on pH was measured in collagen fibres treated with SGTS in vivo and retreated with AuCl$_3$ in vitro (Fig. 4). The $T_s$ values were higher, especially in acid media, in fibres exposed to the combined SGTS and AuCl$_3$ treatment than in those treated with AuCl$_3$ only.

(2) Swelling.—Figs 5 and 6 (overleaf) show that, after 3 weeks' and 5 weeks' administration of SGTS to the experimental animals, the swelling was typically limited, while in the control specimens it was unlimited.

The differences observed decreased with time, obviously because the concentration of cross-linkages also increased with ageing in the collagen of the control animals. The collagen treated with
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Fig. 8.—Contraction-relaxation ageing the administration for It increased.

while contraction and relaxation increase due to cross-linkages.

Fig. 7.—Contraction-relaxation after 2 weeks' treatment with SGTS constant load.

The contraction period was prolonged with SGTS administration for 3 and 5 weeks (Figs 8 and 9), and while the contraction period likewise increased with ageing the differences from the control values also decreased. It may be deduced that, in contraction and relaxation as well as in swelling, the increase in cross-linkages due to ageing tended to mask an increase due to binding of gold.

Fig. 8.—Contraction-relaxation after 3 weeks' treatment with SGTS.

Fig. 9.—Contraction-relaxation after 5 weeks' treatment with SGTS.

Discussion

Schmitt, Hall, and Jakus (1945) first used heavy metals for staining collagen for electron microscopy. According to many authors, the phosphotungstic acid reacts with basic groups of amino acids, i.e. with the quanidine group of arginine and with the ε-amino group of lysine; on the other hand uranyl acetate and chromium react with carbonyl groups of glutamic and aspartic acid (Grassmann, 1960).

To the best of our knowledge the present investiga-

TABLE

EFFECTIVE DEGREE OF CROSS-LINKAGE CONCENTRATION (ν (MOLES PER CC)) AND SHRINKAGE TEMPERATURE (T) OF RAT TAIL TENDON COLLAGEN (MEASURED IN A BUFFER OF pH 7.0)

<table>
<thead>
<tr>
<th>Series</th>
<th>ν (Moles per cc)</th>
<th>T (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>62</td>
</tr>
<tr>
<td>3 weeks’ gold therapy</td>
<td>1.13</td>
<td>64</td>
</tr>
<tr>
<td>0.1 M AuCl₃ tanning in vitro</td>
<td>2.53</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>75.7</td>
<td>87</td>
</tr>
</tbody>
</table>

gold in vitro did not swell at all and, therefore, it could not be tested by this method.

(3) Contraction and Relaxation

(a) Constant Load.—Slight changes in collagen structure induced by early injections of SGTS could be detected by the contraction and relaxation of swollen fibres under a constant load. Fig. 7 shows obvious changes even after 2 weeks' SGTS treatment.
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REFERENCES


Absorption de l’or par el collagène au cours de la chrysothérapie

RÉSUMÉ
Les auteurs décritent la coloration et le tannage in vivo du collagène par l’or. Après l’administration intra-vitale pendant 8 semaines de composés univalents d’or aux rats, les micrographies électroniques des fibres du collagène du tendon de la queue accusèrent quatre bandes de coloration par période. La chrysothérapie amena une augmentation de la température de contraction du collagène du tendon de la queue du rat ainsi qu’une diminution de la capacité de gonflement et une prolongation de la période de contraction et de relaxation. Conformément à l’équation de Flory, la concentration des linkages croisés augmenta après le traitement par l’or.

Absorción de oro por el colágeno durante la crisoterapia

SUMARIO
Los autores describen la coloración y el curtimiento in vivo del colágeno por el oro. Después de la administración intra-vitale durante ocho semanas de compuestos univalentes de oro a ratas, las micrografías electrónicas de las fibras del colágeno del tendón de la cola acusaron cuatro bandas de coloración por período. La crisoterapia causó un aumento de la temperatura de contracción del colágeno del tendón de la cola de la rata así como una diminución de la capacidad de entumecerse y una prolongación del período de contracción y de relajación. En conformidad con la ecuación de Flory, la concentración de los linkages cruzados aumentó después del tratamiento áurico.

The uptake of gold by collagen provides the first demonstration by electron microscopy of intra-vital collagen staining by gold.

Our results suggest that the treatment of collagen with gold causes an increase in cross-linkages. This phenomenon is called "tanning" in industry, and we think that the same term may be used for the in vivo reaction of gold with collagen.

As gold forms complex cations less readily than anions, it is probable that the gold tanning mechanism will differ from chromium tanning in which the main agents are complex cations preferentially combining with the protein (Gustavson, 1956). The structure and reactivities of auro- and auricomplexes have not been studied in detail like the complexes of chromium, and little is known about the various interactions of gold complexes with protein molecules. Anionic complexes of gold are more stable than cationic complexes, and it may, therefore, be assumed that they will participate in gold tanning. This view is also supported by the fact that the slope of the $T_2$ versus pH curve in the case of gold is the reverse of that in the case of chromium and zirconium which are known to tan in the form of cationic complexes (Somerville, 1958). It is noteworthy that, when SGTS is applied in vitro, there is neither an increase in structural stability nor a staining of electron microscopic specimens. A comparison of the dependence of $T_2$ on pH in fibres tanned in vitro by trivalent gold with that in fibres tanned intra-vitally and subsequently extra-vitally suggests that cationic complexes can also play a role in these reactions. The main differences are seen in acid media (Fig. 4).

It is hoped that this example of the reaction of gold with collagen may help to elucidate the mode of action of gold compounds in gold therapy.

Summary

The authors describe the in vivo staining and tanning of collagen with gold. After 8 weeks' intra-vital administration of a univalent gold compound to rats, electron micrographs of tail tendon collagen fibres show four staining bands per period. Gold therapy caused an increase in shrinkage temperature of rat tail tendon collagen as well as a decrease in swelling ability and a prolongation of the contraction and relaxation period. In accordance with Flory's equation, the concentration of cross-linkages increased after gold treatment.
Uptake of gold by collagen in gold therapy.

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Ann Rheum Dis 1965 24: 378-381
doi: 10.1136/ard.24.4.378

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