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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 Wister 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 3μ 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:

(*i*) 1·0 per cent. solution of SGTS;

(*ii*) 0·1 or 0·001 M gold trichloride ($AuCl_3$) 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 (*p*H 2·3; $\mu = 0{\cdot}0125$) by the following quantitative methods:

(1) The shrinkage temperature (*Ts*) was measured microscopically according to the method of Borasky and Nutting (1949), with the fibre submerged in the Britton-Robinson buffer solution, at *p*H's ranging from 2·0 to 10·0 ($\mu = 0{\cdot}0125$);

(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-vitally to the same extent. Well-stained fibres and those showing only poor structure occurred in the same specimen.

* 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.

‡ $f/A_o = \nu RTv_r^{\frac{1}{3}}(\alpha - \alpha^{-2})$,
When f/A_o = the force used and related to the cross section unit of the swollen fibre,
R = gas constant,
T = absolute temperature,
α = relative prolongation of the fibre,
ν = effective degree of cross-linkage (moles per cc.),
v_r = volume ratio of the dry polymer in the swollen sample.

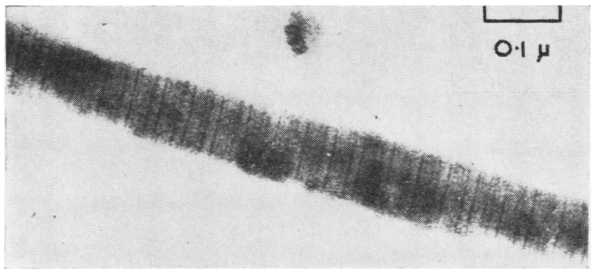

Fig. 1.—Electron micrograph of rat tail tendon collagen stained intravitally with gold thiosulphate.

(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 *p*H values above 4·0, and with 0·001 M $AuCl_3$ treatment at *p*H 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 *p*H 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.

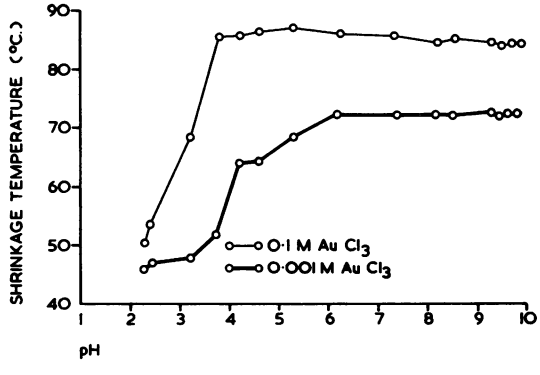

Fig. 3.—Shrinkage temperature (T_s) after gold trichloride ($AuCl_3$) treatment *in vitro*.

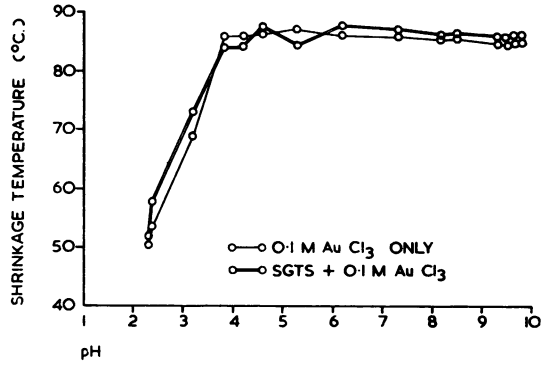

Fig. 4.—Shrinkage temperature (T_s) after SGTS treatment together with 0·1 M $AuCl_3$ *in vitro* and after 0·1 M $AuCl_3$ only *in vitro*.

(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

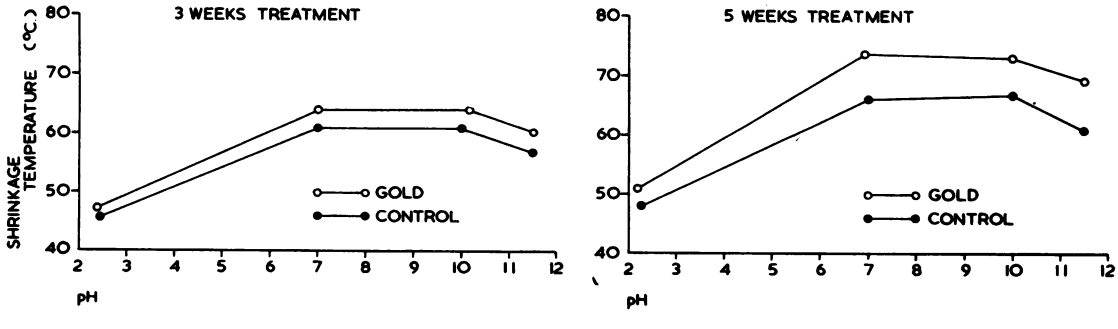

Fig. 2.—Shrinkage temperature (T_s) after sodium gold thiosulphate (SGTS) treatment *in vivo*, after 3 and 5 weeks.

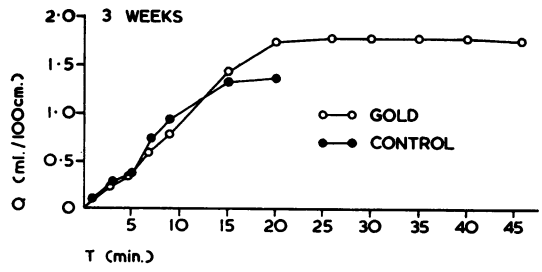

Fig. 5.—Swelling after 3 weeks' treatment with SGTS.

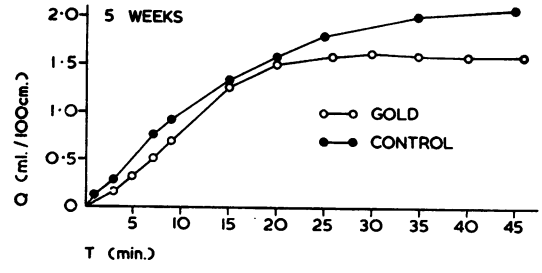

Fig. 6.—Swelling after 5 weeks' treatment with SGTS.

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.

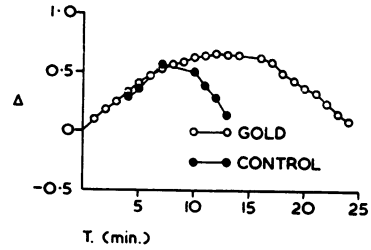

Fig. 7.—Contraction-relaxation after 2 weeks' treatment with SGTS constant load.

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.

(*b*) *Variable Load.*—Contraction and relaxation under a variable load were measured in collagen specimens treated with gold *in vivo* and *in vitro*. According to the results of the Flory's equation, the molar concentration of cross-linkages doubled after 3 weeks' administration of SGTS, and increased 50-fold after *in vitro* treatment with $AuCl_3$ (Table).

TABLE
EFFECTIVE DEGREE OF CROSS-LINKAGE CONCENTRATION (ν (MOLES PER CC.)) AND SHRINKAGE TEMPERATURE (T_s) OF RAT TAIL TENDON COLLAGEN (MEASURED IN A BUFFER OF *p*H 7·0)

Series	ν (Moles per cc.)	T_s (C.)
Control	1·13	62
3 weeks' gold therapy	2·53	64
0·1 M $AuCl_3$ tanning *in vitro* ..	75·7	87

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-

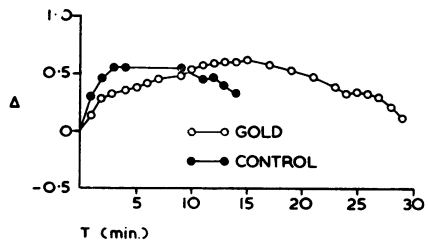

Fig. 8.—Contraction-relaxation after 3 weeks' treatment with SGTS·

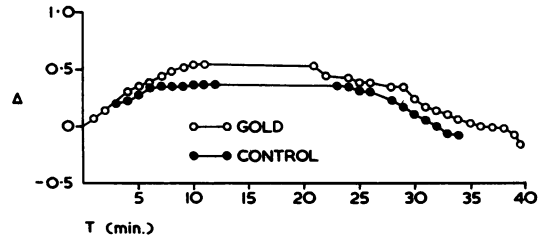

Fig. 9.—Contraction-relaxation after 5 weeks' treatment with SGTS

tion 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 auri-complexes 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_S versus *p*H 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_S on *p*H 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.

REFERENCES

Borasky, R., and Nutting, G. C. (1949). *J. Amer. Leather Chemists Ass.*, **44**, 830.

Delory, G. E., and King, E. J. (1945). *Biochem. J.*, **39**, 245.

Flory, P. J. (1953). "Principles of Polymer Chemistry". Cornell University Press, Ithaca, N.Y.

Grassmann, W. (1960). *Svensk. kem. T.*, **72**, 275.

Gustavson, K. H. (1956). "The Chemistry of Tanning Processes". Academic Press, New York.

Lande, K. (1927). *Münch. med. Wschr.*, **74**, 1132.

Pick, E. (1927). *Wien. klin. Wschr.*, **40**, 1175.

Schmitt, F. O., Hall, C. E., and Jakus, M. A. (1945). *J. appl. Physiol.*, **16**, 263.

Somerville, I. C. (1958). *In* "The Chemistry and Technology of Leather", vol. 2, by F. O'Flaherty, W. T. Roddy, and R. M. Lollar. Reinhold Publishing Corporation, New York.

Absorption de l'or par le collagène au cours de la chrysothérapie

RÉSUMÉ

Les auteurs décrivent 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 relâchement. Conformément à l'équation de Flory, la concentration des linkages croisés augmenta après le traitement par l'or.

Absorpción 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-vital 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.