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# *Studies on "Bluing Effect" in the Petals of Red Rose, IV. Calcium in the Blue Spherical Body.*

By HITOSHI YASUDA

Department of Biology, Faculty of Science,
Shinshu University.
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**Summary**

Both the localization of calcium within the blue spherical body presented in the upper epidermal cells, and the role of the element in this body were studied. The upper epidermis of the bluing petals of a rose cultivar, cl-Crimson Glory, was fixed with 10% neutral buffered formalin, and paraffin sections were prepared. The region corresponding to the central layer of the spherical body was clearly stained red with alizarin red sulfonate under pH 5.5. In the bluing petals treated with EDTA, the central layers of the bodies were not found and the body looked somewhat ring-like. These results show that the calcium localizes within the central body, (a) making a salt or being weakly bound with a tannic substance within the body, and (b) playing a role in making the layer rather rigid.

In previous papers (YASUDA ; 1970, 1974) it was shown that the petals of red roses exhibiting the bluing effect possess the blue spherical body within their upper epidermal cells, and it was also shown that the formation and the structure of this body were observed to have some similarities with the tannin vacuole of the motor cells of *Mimosa*, found by TORIYAMA (1952, 1954). In the series of TORIYAMA's studies on *Mimosa*, there was a report that calcium was localized on the surface of the tannin vacuole and took an important role in keeping the vacuole spherical (1972).

The present study was designed to settle the problem whether calcium exists within the spherical bodies of the rose petals.

When the paraffin sections of the upper epidermal cells of bluing petals were treated with alizarin red sulfonate under pH 5.5, the central layer of the spherical body was clearly stained red (Fig. 1). TORIYAMA *et al.* (1972) pointed out that the alizarin red sulfonate is a specific reagent for detecting calcium which is ionizable or weakly bound in the plant tissues, provided it is applied under pH 5.5. Thus, it seems reasonable to assume that calcium, which is ionizable or weakly bound,

is located within the central layer of this spherical body.

When the paraffin sections were incinerated, the spodograms obtained were the same as those reported previously by YASUDA (1973). Unfortunately, the brownish red color of ferric oxide precluded the recognition of the presence of calcium. But when the incinerated sections were treated with diluted sulphuric acid, the brownish-red color of ferric oxide rapidly disappeared and typical crystals of calcium sulphate became visible (Fig. 4). Even in this observation, the definite localization of calcium was not determined. Thus, whether the strongly bound calcium is present or not within the body, remains uncertain.

When the upper epidermis was treated with EDTA, both the central layers and the outermost layer of the spherical body disappered and the body looks somewhat ring-like (Fig. 2 A, B). In the epidermis treated with EDTA, the color reaction due to alizarin red sulfonate was no longer present in the central layer (Fig. 3). This can be interpreted as an indication that the central and outermost layers are removed by the elimination of calcium. In the outermost layer, the strongly bound calcium may be present.

In the previous paper (YASUDA, 1974), it was shown that the central layer of the body abounds in the tannic substance. Hence, a possible explanation for the results presented here with EDTA, is that calcium forms a salt or weakly binds with the tannic substance, playing an important roll in making the central layer of the body rigid.

TORIYAMA *et al* (1972) reported that calcium is localized on the surface of the tannin vacuole of *Mimosa*, and that the shape of the tannin vacuole varies when the element is eliminated from the surface.

In the present material, on the contrary, calcium is localized in this central portion of the body, and its shape does not change by the elimination of the element with the EDTA treatment. So, it is assumed that the spherical body in the present material is rather rigid. From this view point, the present author should like to say that the spherical body present in the rose petals is unsuitable to be called "a tannin vacuole", but should rather be termed "a tannic body".

## Experimental

1) The materials. The petals strongly exhibiting the bluing effect were collected from a red rose cultivar, cl-Crimson Glory, grown outdoors. The upper epidermis was stripped from the petals, and used as the source material for the histochemical observations.

2) Treatment with EDTA (ethylendiamine tetra-acetic acid). Disodium salt of ethylendiamine tetra-acetic acid was added to 0.1 N phosphate buffer (pH 4.0) to make a 0.02 M solution. The upper epidermis of the petals was soaked in this

solution for about 18 hours. The 0.1 N phosphate buffer which did not contain EDTA was used as a criterion.

3) The upper epidermis, fresh or EDTA treated, was fixed with 10 % neutral buffered formalin at room temperature for 48 hours. After completion of fixation it was washed in running water for several hours and dehydrated through the butylalcohol series and embedded in paraffin. Paraffin blocks were cut 20 micra in thickness. The sections were stained with either fuchsin solution (1 g in 100 ml of 50 % alcohol, staining interval : 30 minutes) or alizarin red sulfonate solution (described below).

4) The alizarin red sulfonate staining. 1 % of alizarin red sulfonate was made up in distilled water and then brought to pH 5.5 exactly with 2 % aqueous $NH_4OH$. The paraffin sections were stained with this solution for 2 hours.

5) The microincineration method. The microincineration method was follows according to the procedure described by TARAO (1957), TORIYAMA *et al* (1962), YASUDA (1973). Briefly, this procedure is as follows : The paraffin sections (10 micra in thickness) were incinerated in a small quarty tube electric furnace after the paraffin was removed in benzol. The temperature of the furnace was gradually increased as follows :

~ 130° ..... 60 min.
~ 350° ..... 60 min.
~ 520° ..... 20 min.
~ 550° ..... 10~20 min.

The incinerated sections were observed by means of dark field illumination.

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**References**

TARAO, S. (1957) Jour. Fac. Sci. Hokkaido Univ. 13 : 326
TORIYAMA, H. (1952) Kagaku 22 : 543
——— (1954) Cytologia 19 : 29
——— (1962) ibid. 27 : 431
——— and JAFFE, M. J. (1972) Plant Physiol. 49 : 79
YASUDA, H. (1970) Bot. Mag. Tokyo 83 : 233
——— (1973) Jour. Fac. Sci. Shinshu Univ. 8 : 91
——— (1974) Cytologia 39 : 107

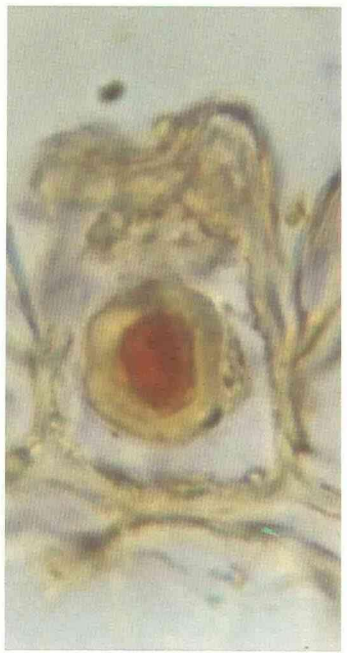

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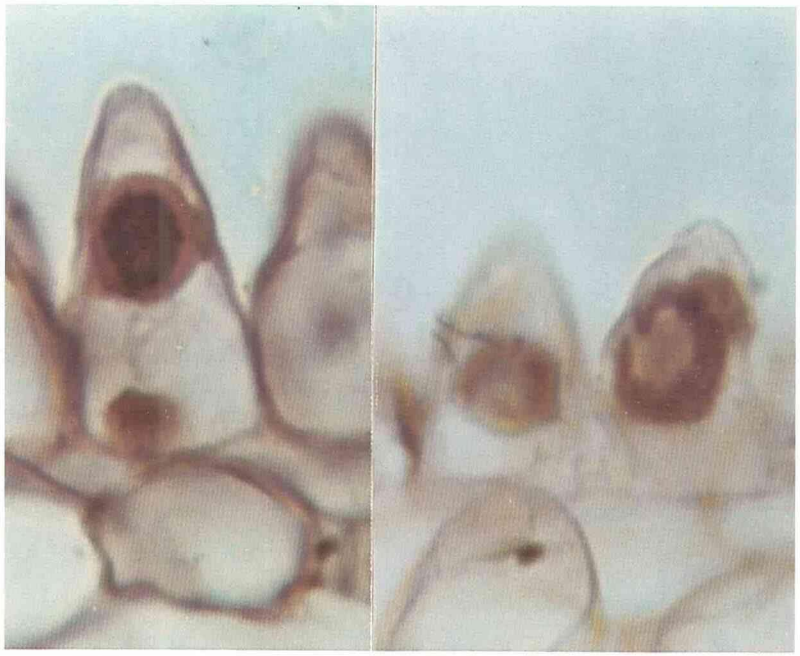
Fig. 1 Upper epidermal cells of rose petals showing bluing effect, fixed in 10% neutral buffered formalin and stained with alizarin red sulfonate (pH 5.5).



2 A　　2 B

Fig. 2 Upper epidermal cells of rose petals showing bluing effect, fixed in 10% neutral buffered formalin and stained with fuchsin. A : Un-treated with EDTA, B : Treated with EDTA.

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Fig. 3 Upper epidermal cells of rose petals showing bluing effect, treated with EDTA, fixed in 10% neutral buffered formalin and stained with alizarin red sulfonate (pH 5.5).

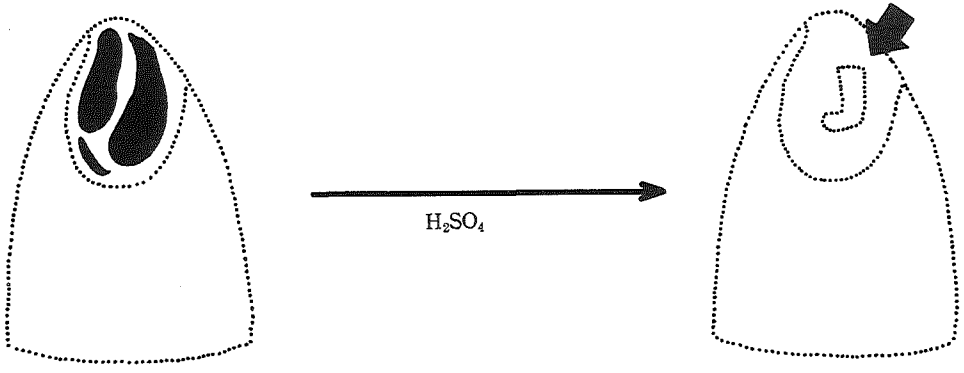


Fig. 4 Spodograms of upper epidermis of rose petals showing bluing effect, fixed in Scott's solution, before and after treatment with diluted $H_2SO_4$. The black areas indicate the exhibition of brownish red color of ferric oxide, and the arrow shows the crystal of calcium sulphate.