# Biological Studies on Cortisone in Mice[1]

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The reported dramatic effects of cortisone in clinical application on such a varied group of disease entities as rheumatic fever, rheumatoid arthritis, lupus erythematosis, asthma, and leukemia have led us to study the physiological mechanism of its action. The present report concerns itself with experimental observations in mice on the influence of cortisone on the formation of granulation tissue (its initiation and continued development), the activity of the reticuloendothelial system, and the formation of acute inflammatory exudate.

*Influence of cortisone on granulation tissue.* Ragan *et al.* (*2*) demonstrated an inhibitory action of cortisone on granulation tissue production in surface wounds on the ears of 6 rabbits. Histologic examination was made late in the process of wound healing, i.e., 5th and 8th days, postoperatively. Similar effects were not obtained by these investigators in rats. In our studies we examined the process of granulation tissue formation from its initiation, as well as the action of cortisone on granulation tissue already well along in formation.

Forty Swiss albino mice (20–25 g) from a bartonella-free strain, were bacteriologically controlled for enteric and respiratory latent infection, housed in individual cages, and kept under controlled conditions.

Following standard surgical techniques, wounds were made with a special instrument that simultaneously produces 2 circular wounds of uniform diameter (0.5 cm) and depth, including the skin and subcutaneous tissue down to the fascial plane of the dorsal muscles.

Based on weight distribution and sex, the mice were divided into a control group of 20 mice (40 wounds), injected subcutaneously with the diluent twice daily, and a treated group of 20 mice (40 wounds), injected subcutaneously with 1.0 mg of cortisone twice daily. Injections were started 24 hr prior to wounding and continued until 4 hr prior to sacrifice in each case.

Four mice in each group were sacrificed on the 1st, 2d, 3rd, 4th, and 5th days following wounding. The total cortisone dose for each mouse in the groups as sacrificed was 4, 6, 8, 10, and 12 mg, respectively. The wounds were completely excised, formalin-fixed, sectioned, and stained with H. & E. and toluidine blue. Complete autopsy was performed on each animal.

Microscopic examination of these wounds revealed a complete suppression of all elements in wound healing of the cortisone-treated group as compared with the control group. Wounds examined after 24 hr (8 wounds in each group) revealed an almost complete lack of exudate and fibrin in the cortisone-treated group. All cellular elements were markedly diminished. As the days progressed, comparable studies revealed very little new capillary formation, sparse fibroblastic proliferation, and scant ground substance present in the cortisone-treated group. By the 5th and final day of this study, the majority of the wounds in the control group showed considerable compact, well-vascularized granulation tissue—almost, if not completely, covered with epithelium—whereas in the cortisone-treated group scant collections of fibroblasts were present. In some of the cortisone-treated animals, however, epithelization was complete, and bare adipose tissue surfaces were practically covered (Fig. 1, *A–D*). The effect on the healing of wounds was in many respects similar to that seen in vitamin C depletion, in particular, the absence of ground substance as demonstrated by the toluidine blue stain.

*Influence of cortisone on existing granulation tissue.* In several instances, clinical investigators have referred to an alleged fibrolytic activity of cortisone to account for beneficial effects in arthritis and for harmful effects on pulmonary tuberculosis. To determine whether such a fibrolytic property exists, 20 mice from the same herd were wounded as described above and divided into 2 groups of 10 mice each. Seventy-two hr after wounding, the treated mice were injected subcutaneously with 1.0 mg of cortisone twice daily; control mice were given the diluent only.

In each group, mice were sacrificed as follows: 3 mice (6 wounds) on the 5th and 3 on the 6th day after wounding; 4 mice (8 wounds) on the 7th day after wounding. The total cortisone administered per mouse was 4.0 mg, 6.0 mg, and 10.0 mg, respectively.

Comparative histologic examination of the wounds did not reveal any significant quantitative or qualitative differences in the composition of the granulation tissue compared on the basis of new capillary formation, intercellular substance, fibroblastic proliferation, and compactness of the granulation tissue. There was no evidence of lysis of already existing granulation tissue.

*Influence of cortisone on the phagocytic action of the reticuloendothelial system.* Fourteen mice selected from the same strain and herd were separated into 2 equivalent groups and pretreated for 2 days with 1.0 mg of cortisone twice daily; the control animals received equal volumes of the diluent. This injection treatment was maintained for each mouse until the day of sacrifice. On the 3rd and 4th days of injection, all mice in both groups were injected with 0.5 ml of carbon particles (India ink) intraperitoneally. On the 5th day, 4 hr after injections of cortisone and diluent, all mice were sacrificed.

The peritoneal cavity and visceral organ surfaces of each of the cortisone-treated mice were diffusely covered with grossly visible carbon particles throughout. The control mice showed only occasional foci of carbon particles on the surface of the liver or spleen, and most of the carbon particles were found in the omental, mesenteric, and retroperitoneal regions. The superior mediastinal lymph nodes of the untreated mice were readily identified by their content of carbon particles,

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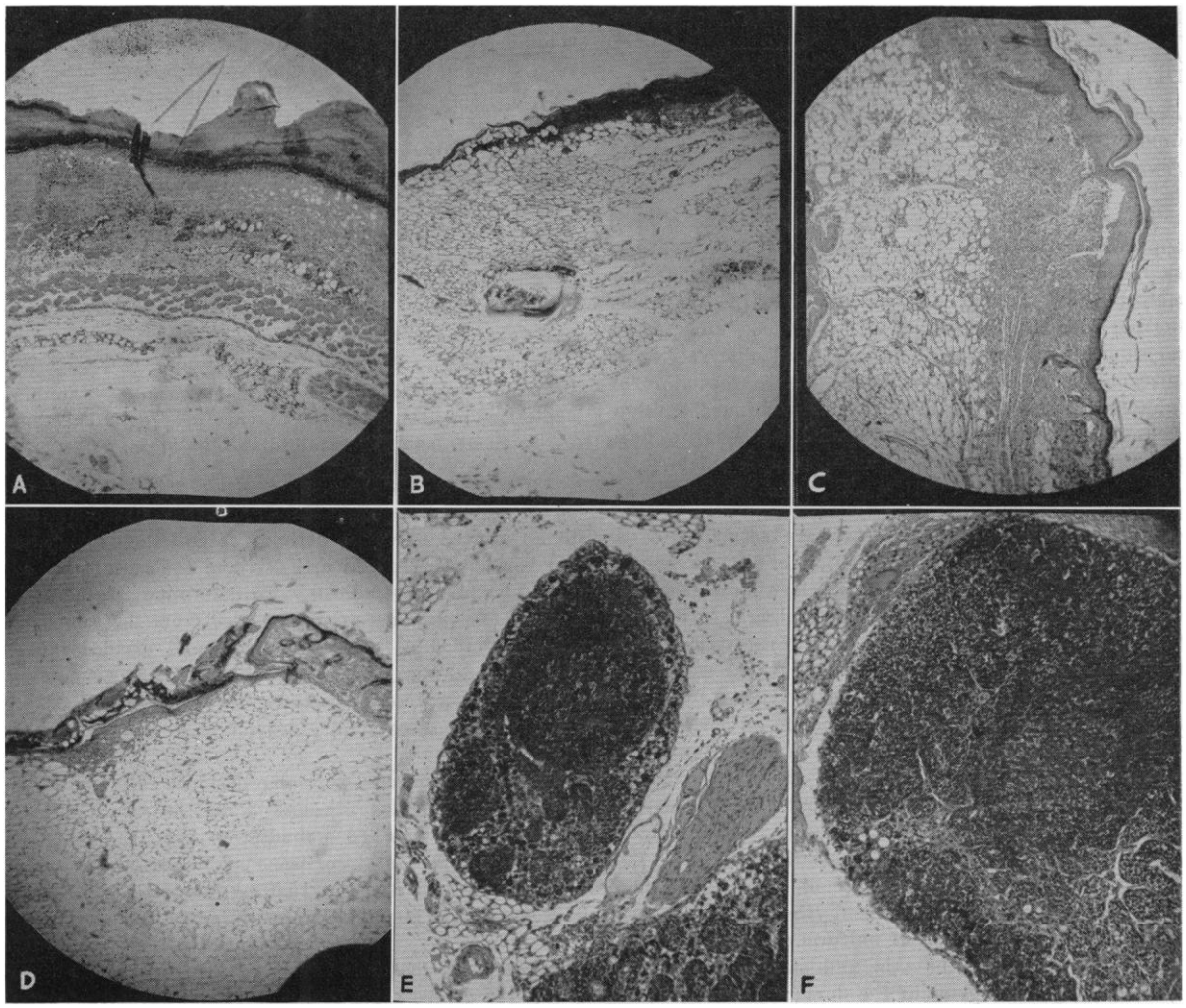


FIG. 1. *A*, photomicrograph of 24-hr wound in control animal, showing usual amount of fibrin and cellular reaction; *B*, 24-hr wound in cortisone-treated animal, showing paucity of fibrin and cellular reaction; *C*, 5-day wound in control animal, showing abundant compact granulation tissue; and *D*, 5-day wound in cortisone-treated animals showing scarce granulation tissue. *E*, mediastinal lymph node in control animal showing carbon particles in macrophages; *F*, mediastinal lymph node in cortisone-treated animal showing no carbon particles in macrophages.

whereas in the cortisone-treated mice the nodes were difficult to locate and contained no carbon particles, except in one instance in which a very small amount of carbon was seen. These gross findings were confirmed by microscopic examination (Fig. 1, *E*, *F*).

An interesting side observation was that the spleens of the cortisone group were 1/5 the size of those in the control group (*1*).

*Influence of cortisone on acute inflammatory exudate.* Clinical reports on the action of cortisone in affecting the resolution of the acute inflammatory exudate of pneumonia prompted us to investigate the development of acute inflammatory exudate in normal cortisone-treated mice.

Ten mice from the same herd and strain as before were divided into 2 groups of 5 each. Pretreatment with cortisone (1.0 mg twice daily) and, in the case of control mice, with equivalent volumes of diluent, injected subcutaneously, was given for 3 days. Total cortisone injected was 8.0 mg/mouse. Four hours after the 7th injection of cortisone and diluent, each mouse was given 0.2 ml of turpentine intracutaneously on a prepared and marked area of the abdominal wall. One additional injection of cortisone and diluent was administered 4 hr later. All animals were sacrificed 18 hr later. The area of turpentine injection was excised, sectioned, and examined microscopically. There were no differences observed in the acute inflammatory exudate between the cortisone-treated and control mice.

Unquestionably, cortisone inhibits the formation of granulation tissue. It appears that the effect is rapid after administration and is of short duration. Significant interference with the formation of granulation tissue occurs only when the cortisone is administered during the early stage of initiation of the repair stimulus. No appreciable effect is noted on already existing granu-

lation tissue. This tends to vitiate usefulness of cortisone as a fibrolytic agent in diseases such as silicosis, scleroderma, etc. It would be harmful to administer cortisone alone in diseases such as tuberculosis, where the fibrous tissue response must not be inhibited.

Cortisone treatment in mice results in a definitely retarded macrophage response. Whether this is due to the diminution of activity of the individual cell or to the depletion or mobilization of the total number of cells could not be determined in the above experiment. Some evidence of total depletion is indicated in our finding (*1*) that administration of cortisone results in a marked reduction of the total cellular content of the spleen. The retardation of the macrophage response may result in a potentially undesirable situation in certain disease processes, e.g., in tuberculosis. Another factor in the retardation of the removal of carbon particles may be due to the stimulation of antihyaluronidase activity by cortisone. This has been reported by Seifter and associates (*3*).

No effect of cortisone on the early formation of acute inflammatory exudate in response to turpentine was noted. This experiment was limited to the early response and did not control the rate of resolution. This question is at present under study by us.


References

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## Additional Confirmatory Evidence of the Rediscovery of the Old Italian Varnish


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In an investigation to rediscover the "lost art" of making the old Italian varnish used centuries ago by the celebrated violinmakers, Stradivarius, the Amati, and others, it was learned from spectrographic analyses (*3–5*) that a number of elements were frequently present in their varnish. The principal elements in the brown varnish were aluminum, iron, silicon, sodium, calcium, magnesium, lead, and manganese, in the order named. Aluminum, iron, and silicon were present in *all* twelve specimens of brown varnish analyzed. The presence of every element, excepting silicon, has been explained, but the unexpected and constant appearance of silicon was perplexing. Moreover, any valid rediscovery of the so-called secret of Stradivarius must account for all the facts. A satisfactory explanation for the presence of silicon thus became necessary.

In 1946 the writer (*2*) proposed that the old Italian violinmakers used metal rosinates in their varnishes and that they, or the alchemists and apothecaries of their time, could have prepared these resins in the following manner. Ordinary rosin was first dissolved in potassa lye extracted from wood ashes, the principal source of alkali in those bygone days. The metal rosinates were then obtained simply by precipitation with a solution of a metal salt such as alum (aluminum-potassium sulfate) or copperas (ferrous sulfate). Since silica or silicates are always present in wood ashes, it was suspected that these might also be the source of the silicon found in the varnishes. The silicon would thus serve as a telltale element that should shed light on the method and the materials employed in making the old Italian varnish.

*Theoretical considerations.* The extraction of alkali from wood ashes is almost a forgotten art, so it may be of interest to delve briefly into the process. The soluble alkali in the ashes was extracted by three methods: (*a*) extraction with water, which yielded the alkalis in the form of carbonates; (*b*) subsequent addition of lime to this solution, which yielded a stronger lye, but which required two operations or filtrations; and (*c*) extraction of the wood ashes with water and lime (milk of lime), which yielded the lye solution readily with only one filtration or decantation. It would be necessary to boil the carbonate solution obtained from method (*a*) to dissolve rosin; the stronger lyes (potassium and sodium hydroxides) dissolve rosin in the cold. Thus, method (*c*) is simple and direct and is the most likely and logical method that the ancients might have used to prepare their lye solutions to dissolve rosin.

The alkali hydroxides formed in method (*c*) should also dissolve some of the silica present in the wood ashes, converting the silica into soluble alkali silicate. The silicate would in turn be precipitated when the alkali rosinate solution reacted with the metal salt solutions in the precipitation of the metal rosinates. The chemical reactions are as follows:

$$K_2CO_3 + CaO + H_2O = CaCO_3 + 2KOH$$
$$SiO_2 + 2KOH = K_2SiO_3 + H_2O.$$

*Experimental results.* Only materials and methods fully justified by recorded writings preceding and contemporary with the period in which the old Italian varnish was in existence (A.D. 1550–1750) were used in this research. Well-burned wood ashes were digested overnight with water to which lime (CaO) had been added, and the mixture was filtered through cloth which yielded a water-white filtrate. Freshly powdered rosin in small amounts was then added to the filtrate in the cold, with occasional shaking until an excess of rosin remained; upon standing a few days, clear, amber-colored solutions were obtained; undissolved pieces of rosin were filtered off and weighed. The results of several extractions are summarized in Table 1.

An alum solution or a solution of alum and copperas was then added to the alkali rosinate solution until an excess of the precipitant was present. The mixture was heated on a water bath, which caused the precipitated metal rosinate to coalesce and expedited filtration through cloth. The resulting air-dried resins were freely soluble in turpentine, and these solutions gave glass-clear films upon drying.


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