Effect of Ascorbate on Healing of Poultry Bruises.* (26887)

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Acceleration of the healing rate of a bruise depends on many factors(4,5) which affect in some way the physical and biochemical reactions of the tissue during the first and second phases of healing. The intense detoxication, protein degradation and renewed biosynthesis, especially that of protein, are among the important factors involved in healing of traumatic injury.

The importance of Vit. C in wound healing is well known. Protein changes, such as collagen synthesis(1,3,13), degradation of hemoglobin(10,11), and formation of bilirubin(4) possibly through reduction of biliverdin, seem to require an abundant supply of ascorbic acid above that usually synthesized by the broiler under normal conditions of growth and handling. This possible need of ascorbate by the injured tissues may be a therapeutic agent to decrease the overall loss and damage due to bruising in poultry. It has been estimated that the loss and damage to the commercial broiler industry due to bruising amounts to 2 million dollars annually in the State of Georgia alone, yet relatively little is known as to how these bruises are inflicted, possible practical and simple means of minimizing the extent of damage, or accelerating the rate of healing of these injured tissues.

Therefore, the studies to be reported here were conducted to investigate the effect of ascorbate on rate of healing and on some biochemical and physical changes occurring in experimentally-inflicted poultry bruises.

Materials and methods. Bruising and sampling. Eight- to 10-week-old birds kept in constant temperature houses were bruised using a standard technic(6). Control and bruised tissues were excised and subjected to various chemical and biochemical analysis as described previously(4,6,7). *Ascorbate.* Total ascorbic acid, defined as the sum of L-ascorbic, dehydroascorbic and 2,3-diketo-l-gulonic acids, was determined by the 2,4-dinitrophenylhydrazine method of Roe and Kuether(16) as modified by Schaffart and Kingsley(17). Ascorbic acid, *i.e.*, the reduced form alone, was determined by the agar diffusion method of Chaiet and Chaiet(2).

Results and discussion. Blood ascorbic acid levels as affected by environmental temperature and bruising. The blood of normal (non-bruised birds 8-10 weeks old) kept or raised at 7.2, 21, and 30°C was assayed for its total and reduced ascorbic acid contents. Another group of birds kept at the same temperatures were bruised and their blood examined 48 hours after contusion. The level of total ascorbic acid in the blood of the control was higher in chickens kept at 7.2 or 21°C than those raised at 30°C (Table I). The reason for this difference is not clear. Ascorbic acid concentration in the blood of birds, 48 hours after bruising, was much lower than that of the non-bruised birds which may indicate a depletion of the ascorbate probably due to its rapid utilization by the injured birds for degradation of hemoglobin present in the bruised area(11), for formation of collagen fibers of the damaged areas(1,3,13,15), or for synthesis and incorporation of "active" hydroxyproline(14). Depletion of ascorbate in the blood of the bruised birds confirms the findings reported by Merezhinskii, Yadvinskaya, and Ivanova (12) on skin burns.

Effect of sodium ascorbate on healing.

TABLE I. Effect of Environmental Temperatures and Bruising on Total Ascorbic Acid Levels in Blood of Chickens.

No. of birds	Temp., °C	Ascorbic acid conc., mg/100 ml Control*	Bruised†
64	7.2	4.80 ± .82	2.60 ± .60
23	21	4.00 ± .80	2.50 ± .60
66	30	3.10 ± .90	1.50 ± .60

* Control non-bruised birds.
† 48 hr post bruise.

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TABLE II. Effect of Injecting Na-Ascorbate on Healing Time and Bilirubin Detected in Poultry Bruises.*

No. of birds	Treatment received (daily)	Healing times (hr)	Bilirubin detection after 12 hr†
12	.2 mg	72 ± 12	slight
12	2 mg	60 ± 12	positive
12	20 mg	72 ± 12	positive
12	No treatment (control)	96 ± 12	negative

* The birds were kept at 7.2°C.
† Conducted on excised tissues.

After the need of ascorbate by the bruised birds above that normally synthesized was demonstrated we examined the effect of injecting sodium ascorbate at site of the bruise on rate of healing of experimentally-induced poultry bruises. Four groups of birds kept at 7.2°C were bruised. Immediately following bruising and daily thereafter, birds of the first group received intramuscular injections of 0.5 ml aqueous solution containing 0.2 mg Na-ascorbate at the center of the damaged areas. The second and third groups were treated similarly except that concentrations of the injected ascorbate were 2 and 20 mg of ascorbate per 0.5 ml aqueous solution, respectively. The fourth group received no treatment. Morphological examination of the bruises before and after killing and detection of tissue bilirubin or biliverdin, a reflection of hemoglobin degradation(5), served as criteria for the effectiveness of the injected ascorbate in promoting or accelerating the rate of healing in poultry bruises.

The results (Table II) indicate that bruises injected daily with 2.0 mg sodium ascorbate healed at a significantly higher rate than observed in birds treated with 0.2 or 20 mg or non-treated bruises. Healing as detected by gross observation occurred within 60 ± 12 hours post bruising whereas the non-treated bruises healed in an average time of 96 ± 12 hours. Bilirubin was chemically detected by the Fouché reagent in the bruised tissue 12 hours after bruising in Na-ascorbate treated birds but not in non-treated bruises. Fast degradation of hemoglobin and accumulation of bilirubin were observed at site of Na-ascorbate injection as measured spectrophotometrically (Table III).

Effect of force applied. A number of birds, kept at 7.2°C, were divided in 3 series and breast bruised as follows: the first sere were bruised using 1 blow; the second received 3 blows whereas the third received 5 blows. Each sere was again subdivided into 2 groups, A and B; Group A received daily intramuscular injection of 2.0 mg sodium ascorbate in 0.5 ml aqueous solution whereas group B served as non-bruised controls. At intervals bruised birds from each sere and group were examined morphologically before and after killing. The results of this experiment indicated that injection of sodium ascorbate reduced the size of the damaged areas after the first injection as evidenced by a clear zone at site of the injected ascorbate. It is believed that ascorbate favorably influenced regeneration of the damaged tissues probably through activating some enzymes, the synthesis of collagen(1,13) in the bruised areas, or accelerating rate of degradation of extrastromal hemoglobin(4) possibly through

TABLE III. Effect of Sodium Ascorbate on the Extrastromal Hemoglobin and Bilirubin Concentration in Poultry Bruises.

Age of bruise, hr	Concentration in mg/100 g tissue: Hemoglobin Non-treated*	Hemoglobin Treated†	Bilirubin Non-treated	Bilirubin Treated
½	2.0 ± .6	2.0 ± .6	.00	.00
12	7.8 ± .7	8.0 ± .8	.75 ± .25	.8 ± .3
18	10.6 ± 1.0	7.2 ± .8	1.8 ± .40	2.0 ± .40
24	12.8 ± 2.0	6.6 ± .8	2.15 ± .30	4.0 ± .40
48	9.2 ± .8	4.2 ± .6	2.75 ± .40	2.0 ± .40
72	2.3 ± .5	.6 ± .5	1.20 ± .40	.00± .1
96	.6 ± .2	.5 ± .4	.45 ± .3	.00
120	.5 ± .2	.5 ± .4	.00 ± .1	.00

* Non-treated bruised birds. † Birds receiving daily inj. of 2.0 mg Na-ascorbate.

coupled oxidation with ascorbate(10).

Effect of ascorbate on pigments present in poultry bruises. Two groups of birds, kept at 30°C, were bruised using a standard technic. One group received daily injection of 2.0 mg Na-ascorbate whereas the other group served as control birds. At various time intervals, the birds from both groups were slaughtered, the bruised tissues excised, and concentration of the extrastromal hemoglobin, bilirubin, and biliverdin in the tissues determined. The results showed that bruised birds receiving daily injections of the Na-ascorbate had a lower concentration of extrastromal hemoglobin 18, 24, and 72 hours after bruising than bruised birds receiving no treatment. Sodium ascorbate treated birds accumulated a high concentration of bilirubin 24-48 hours post bruising rather than biliverdin which normally accumulated in control bruises in birds kept at 30°C(7). The accumulation of bilirubin by the Na-ascorbate treated birds may be due to reduction of the free biliverdin at the γ-methene bridge. The latter is formed from hemoglobin which appears to undergo scission at the α-methene bridge as described previously (4).

Effect of Na-ascorbate on proteolytic activities of bruised tissues. Two groups of birds kept at 7.2°C were bruised; the first group was injected daily with 2.0 mg Na-ascorbate in 0.5 ml distilled water whereas the second group served as control bruised birds. At various intervals during healing, birds from each of the groups were sacrificed, the bruised tissues excised and assayed for their proteolytic activities using casein as a substrate(8). The results showed that ascorbate-treated birds had a much higher proteolytic activity at both 24 and 48 hours post bruising followed by a considerable decrease after 72 hours (complete healing) compared with the non-treated bruises. The latter had lower activities after 24 and 48 hours post bruising and much higher activity after 72 hours. The early increase in proteolytic enzymes *in vivo* following the injection of Na-ascorbate may be due to activation of these enzymes which could be another factor accounting for the accelerated healing of Na-ascorbate treated bruises. Activation of proteolytic enzymes in rat skeletal muscles by glutathione and by cysteine (reducing and sulfhydryl agents) was reported recently by Koszalka and Miller(9). Zannoni and La Du (18) established that inactive p-hydroxyphenylpyruvic acid oxidase can be reactivated *in vitro* by addition of ascorbic acid.

Summary. The level of ascorbate in the blood of normal birds was higher in birds kept at 7.2 and 21°C than in those raised at 30°C. Bruising resulted in a depletion of ascorbate in the blood probably due to utilization by the damaged tissues. Bruises injected daily with 2.0 mg Na-ascorbate healed at a much faster rate than bruises of non-treated birds. This treatment resulted in fast degradation of the hemoglobin present, mostly to bilirubin in birds kept at 30°C which normally accumulate biliverdin. Na-ascorbate reduced the size of the bruise and activated proteolytic enzyme in bruised tissue.

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An Effect of Tolbutamide on Nucleotides of Incubated Whole Human Blood.* (26888)

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Tolbutamide is an oral hypoglycemic agent whose mode of action is still in dispute. If this class of arylsulfonamides acts only in the animal containing some islet tissue(1), then these compounds should have no effect *in vitro* on animal tissue since no insulin-producing tissue would be present to be acted upon. Lundbaek *et al.*(2) noted that tolbutamide added to the isolated rat diaphragm would increase glucose uptake, and Renold *et al.* (3) reported that tolbutamide inhibited ketogenesis by rat liver slices and affected glucose oxidation and lipogenesis from glucose by surviving rat adipose tissue. The present work shows that tolbutamide affects the nucleotide pattern of incubated whole human blood and since this pattern is closely related to glycolysis in the red cell, suggests, but does not explain, an influence on carbohydrate metabolism.

Materials and methods. Blood was collected from 2 donors in such a way that the blood samples contained about 2,000 units of heparin and 350 mg of glucose per 100 ml of blood. Aliquots were placed in 2 tissue culture spinner flasks specially water jacketed to maintain 37° and to one flask in each experiment was added 250 mg tolbutamide (Orinase) suspended in 2.5 ml of saline. Samples were withdrawn from all flasks aseptically at several subsequent time periods and trichloracetic acid extracts were made for subsequent nucleotide fractionation(4).

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Results. In Fig. 1 and 2 are shown the concentrations of hypoxanthine (Hx), inosine monophosphate (IMP), and adenosine mono-, di-, and tri-phosphates, (AMP, ADP, ATP) in the control and tolbutamide flasks for the 2 subjects. Since ATP is the key energy compound and since on breakdown it follows the pattern(5):

$$ATP \rightarrow ADP \rightarrow AMP \rightarrow IMP \rightarrow\rightarrow Hx$$

it is apparent that in both experiments the presence of tolbutamide was associated with some breakdown in ATP and buildup of degradation products. At 24 hours the changes have started and by 48 hours they are easily seen.

Other measurements such as glucose, pH, and plasma hemoglobin were made in both experiments. The pH in the tolbutamide samples was as much as .1 pH unit below the corresponding control pH at intermediate time intervals but both control and tolbutamide samples came to the same pH by 48 hours. Glucose utilization under the two conditions was not different.

Discussion. Since in the mammalian red cell, glycolysis is the sole source of energy, ATP buildup must be directly coupled with glycolysis. The effect of tolbutamide in reducing blood ATP levels *in vitro* means either that tolbutamide slows ATP synthesis or accelerates its breakdown. The latter might suggest activation of "latent ATPase" while the former could be explained as analogous to "uncoupling" in respiratory phosphorylation. Either process could be compatible with essentially no change in glucose utilization in this *in vitro* system but would