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Some Biochemical and Physical Changes Occurring in Experimentally-Inflicted Poultry Bruises.* (26886)

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In previous publications on bruised tissue (9) evidence was presented that the characteristic color alterations in bruised tissue represent definite stages in the various physical and metabolic changes which occur during healing. Furthermore, it was shown that when cattle(10) or chickens(12) were bruised upon a second or third occasion, each successive bruise healed more rapidly than the preceding one. Serological and electrophoretical analyses of rabbit serum following bruising have shown that a humoral factor may be responsible for the accelerated healing of secondary bruises(8) and that injection of streptokinase or trypsin enzymes at site of a bruise enhanced the rate of healing in rabbit bruises(13). Zamecnik, Stephenson, and Cope(21) demonstrated the presence of an amino exopeptidase enzyme in normal lymph, serum, muscle, skin, subcutaneous tissue and erythrocytes of dogs and established that the level of this enzyme rises abruptly in the lymph draining a burned or traumatized tissue. The changes that occur in the concentration of a number of compounds of biochemical interest in the plasma and the damaged tissue following onset of an acute inflammatory response(5), trauma(7,14), or wound(18,20) have been investigated.

The present study was initiated to investigate the proteolytic activities and physical changes that occur at specific time intervals following infliction of bruises in poultry. Some of the factors which affect these changes were also examined.

Materials and methods. Bruising and sampling. Chickens 8 to 10 weeks old and weighing approximately 3 lb were kept in constant temperature houses at 7.2, 21 and 30° C for 5-7 days prior to bruising. The area to be bruised and control (non-bruised areas) were clipped and the birds bruised using a standard technique on the breast or the thigh muscles(12). Control and bruised birds were sacrificed at specific intervals following bruising, and both the bruised and control tissues were immediately excised, sliced, minced, and aliquants used for analyses. *Assay of proteolytic activity.* A homogenate of the bruised or control tissue was prepared at 5° C by blending 25 g of the minced tissue with 75 ml of chilled 0.9% NaCl and removing the insoluble residue (blood cells, nuclei and mitochondria) by centrifugation at about 3° C at 6,000 × g for 20 minutes. The supernatant solutions were assayed for proteinases by the method of Kunitz(16) with 0.5% buffered casein as substrate. The proteolytic activity is expressed herein as optical density (O.D.) increment of the incubated solution for 1 hour minus that of the corresponding blank at zero time. Specific activity was defined as Δ O.D. at 280 mμ per 1 hour per

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mg protein. The latter was determined by the biuret reaction(6) with crystalline egg albumin as the protein standard. *Pigment extraction.* Aliquants of the minced tissue were subjected to various extraction procedures using saline, buffered to pH 7.4 and containing 1% serum albumin, followed by chloroform, and then alcohol to determine the percent hemoglobin, bilirubin, and biliverdin as described earlier(9,10,11,12). The various extracts were examined spectrophotometrically for extrastromal hemoglobin bilirubin, and biliverdin.

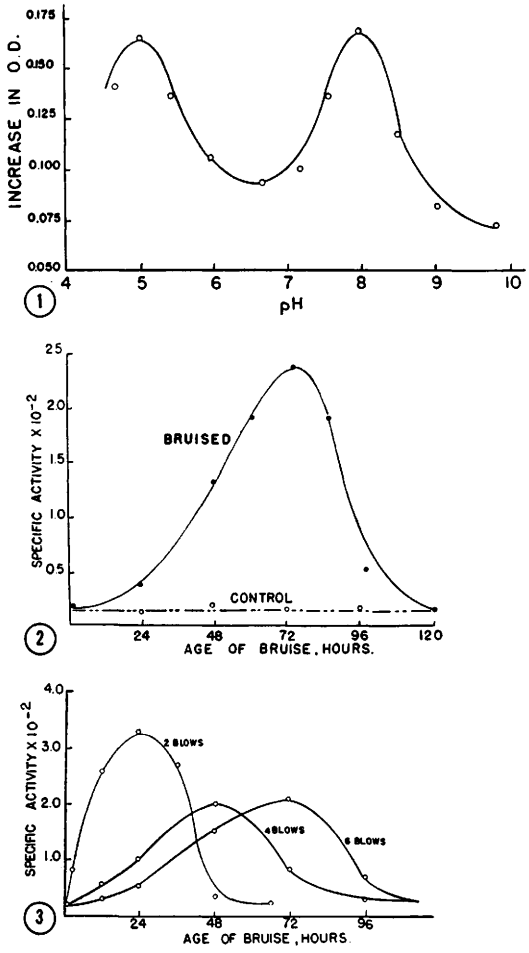

FIG. 1. Hydrolysis of casein by extract of bruised tissue as a function of pH.

FIG. 2. Effect of age of bruise on proteolytic activity present in damaged tissues.

FIG. 3. Effect of severity of bruise on proteolytic activity. Three groups of birds, kept at 7.2°C, were bruised on the breast. The first group received 2 blows, the second 4 blows and the third 6 blows.

Results and discussion. Characterization of proteolytic activity. Fig. 1 shows the effect of pH on hydrolysis of casein by crude extracts of a 2-day-old bruised tissue containing 10 mg protein per ml. The ordinate shows the increase of optical density at 280 mμ for incubation of one hour, and the 2 peaks suggest the presence of at least 2 proteinases in the bruised tissue with pH optima at 5.0 and 8.0, respectively. Heating the bruised tissue homogenate at 80° C for 10 minutes destroyed all the proteinase activity. Action of the plasma cells, the leukocytes, or the autolytic activities of the damaged cells as well as the fluid and serum in the bruised area may be the major sources of these proteolytic activities. Fleisher(4) reported that human leukocytes contain a glycylglycine dipeptidase showing optimal activity at pH 7.7. Koszalka and Miller(15) established that rat skeletal muscle homogenate contained a proteolytic enzyme which readily attacks the endogenous muscle protein at an optimal pH of 8.5 to 9.0. Saline extracts of whole rats and beef lung were found by Dannenberg and Smith(1) to exhibit 2 regions of optimal activity at pH 4.0 and 8.4.

Effect of age of bruise. After the presence of proteolytic activity was established it was decided to examine factors which may affect the proteolysis, optimally active at pH 8.0 in the bruised tissue. Fig. 2 represents typical results of the effect of age and indicates that control and bruised tissue immediately following contusion exhibited the same proteolytic activity. Slight activity was noted 24 hrs after infliction of the bruise, followed by an abrupt increase after 48-72 hours, then a rapid decrease within 72 to 96 hours reaching the control level 120 hours post bruise. Evidence of complete healing, as detected morphologically, was noted from 96 to 120 hours after contusion. The increase in proteolytic activity in the bruised area may be due to the activity of mononuclear phagocytes invading the tissues as well as to the autolysis of the tissue cells. Lymphocytes, monocytes and neutrophils were observed (17) infiltrating livestock bruises one day after bruise infliction and the sarcoplasm exhibited a lytic vacuolization (hydropic de-

generation) 2 days post bruise. Wachestein (19) and Fell and Danielli(3) demonstrated evidence of increased enzymatic activity in neutrophils in inflammatory processes. De Duve(2) reported that the lysosomes particles may be involved in intracellular digestion in connection with phenomena of phagocytosis and physiological autolysis or necrosis.

Effect of severity of bruise. The results (Fig. 3) showed that considerable proteolytic activity was detectable within 6-8 hours and reached a maximum after 24 hours, in a superficial type bruise inflicted by using 2 blows, followed by a rapid decrease. Medium (4 blows) and severe bruises (6 blows) exhibited very slight proteolytic activities within 24 hours following bruising. Then a gradual increase to reach a maximum within 48 to 72 hours was observed, with a gradual decrease thereafter. Healing was detected after 2 to 3 days when chickens were bruised superficially, 4 to 5 days when moderately bruised, and 5 to 6 days when severely bruised.

Effect of successive bruising. It has been shown that each of 3 successive bruises in animals(10) or chickens(12) healed more rapidly than did the preceding one. The factor or factors responsible for this accelerated healing were present, at least in the whole blood, and may be passively transferred to a normal animal by transfusion. It was also thought that this successive bruising might affect the proteolytic activity of the injured tissues. Three groups of birds were used in this experiment. Birds of the first group were bruised using 3 blows; some of the birds were sacrificed at 24 and others at 48 hours. The bruised tissues were excised and assayed for proteolytic activity. The second group was treated similarly except each bird was bruised a second time 2 days later using the same applied force. The third group was bruised on 3 occasions 2 days apart. The results are recorded in Table I and indicate that the tissue extract of birds given 2 or 3 separate bruising treatments was more active proteolytically at 24 and 48 hours than the tissue of birds bruised upon only one occasion. The increased activity in chickens previously bruised once or twice may be a result of activation of proteinase synthesis induced by the presence of some factor in bruised tissue, or it may be a direct response to the stimulated phagocytosis following the first bruise. The significant increase in proteolytic activities may account for the accelerated healing on successive bruises as previously established (10,12).

TABLE I. Effect of Successive Bruising on Proteolytic Activities of Tissue.*

No. of birds	Age of bruise	Specific activity $\times 10^{-2}$* 1st bruise	2nd bruise	3rd bruise
5	24 hr	.3 ± .05	1.0 ± .20	1.3 ± .30
6	48 "	1.3 ± .20	1.7 ± .30	2.0 ± .30

* Specific activity was defined as △ O.D. at 280 mμ/1 hr/mg of protein. This value was found to be 0.2×10^{-2} for control non-bruised tissue.

Physical changes occurring in poultry bruises. The spectrophotometric absorption curves of the buffered saline and chloroform, and alcohol extracts of control and 2-day-old bruise samples secured from broilers, raised at 7.2, 21 and 30° C, showed that the buffered-saline extract of the bruised area contained hematin and hemoglobin whereas the alcohol extract contained a high concentration of biliverdin. The chloroform extract of bruised tissues secured from birds raised at 30° C showed the presence of a significantly low concentration of bilirubin compared with that obtained from birds raised at 7.2 or 21° C. After the presence of hemoglobin, bilirubin and biliverdin were established in bruised tissue, it was decided to follow quantitatively their changes during healing and also examine the effect of environmental temperatures on the pigments present. The results (Table II) indicate that bruised tissue secured from birds raised at 21 or 30° C accumulated extrastromal hemoglobin which increased in concentration to reach a maximum at 24 hours, followed by a rapid decrease to reach the level of the normal tissue 3-5 days postbruise. The predominant bile pigment present as a result of hemoglobin degradation(9) was found to be bilirubin in birds raised or kept at 21° C and biliverdin in birds raised or kept at 30° C. These pigments were absent in the damaged areas immediately fol-

TABLE II. Effect of Environmental Temperature on Pigments Present in Poultry Bruises during Healing.

Age of bruise in hr	No. of samples	Concentration in mg/100 g tissue* Environmental temperature 21°C Hemoglobin	21°C Bilirubin	21°C Biliverdin	30°C Hemoglobin	30°C Bilirubin	30°C Biliverdin
½	14	2.0 ± .6	.00	.00	3.2 ± .5	.00	.00
12	12	7.8 ± .7	.7 ± .3	.5 ± .3	8.6 ± .8	.1 ± .05	.9 ± .2
14	13	8.9 ± .7	1.0 ± .4	.5 ± .3	10.2 ± .8	.2 ± .05	1.1 ± .2
18	10	10.6 ± 1.0	1.8 ± .4	.6 ± .2	12.1 ± 1.2	.4 ± .2	2.0 ± .5
24	13	12.8 ± 2.0	2.2 ± .3	.8 ± .3	14.2 ± 2.3	.6 ± .3	3.0 ± .4
48	16	9.2 ± 1.0	2.8 ± .4	1.2 ± .4	6.3 ± 2.0	.8 ± .2	1.6 ± .4
72	12	2.3 ± .5	1.2 ± .4	.6 ± .2	1.0 ± .4	.1 ± .05	.3 ± .1
96	12	.6 ± .2	.5 ± .3	.1 ± .05	.7 ± .2	.00	.00
120	11	.5 ± .2	.00	.00	.6 ± .1	.00	.00

* Normal breast muscle tissue contained .7 ± .2 mg hemoglobin and .00 bilirubin or biliverdin/100 g tissue.

lowing bruise infliction; increased slightly in concentration after 12-14 hours and rapidly after 18-24 hours to reach a maximum after 24-28 hours. They then disappeared from the bruised areas after 96 hours. The accumulation of biliverdin in bruised tissues in birds kept at 30° C may be due to some fundamental difference in the hemoglobin degradation in general or to the impaired synthesis of reducing compounds such as ascorbate or glutathione.

Summary. Proteolytic activities, detected in experimentally-inflicted poultry bruises, were found to be influenced by many factors such as previous bruising, severity, and age of the bruise. Bruised tissues accumulated extrastromal hemoglobin which was degraded primarily to biliverdin in bruises contused on birds kept at 30° C and to bilirubin in birds kept at 21° C or below.

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