subsequent work in progress is designed to explore them.

Summary. 1. Poliomyelitis virus of high titer in the form of a freshly filtered tissue culture preparation, can be inactivated by ultraviolet irradiation in centrifugal filmers. 2. Inactivation of the virus preparations by a combination of ultraviolet irradiation and exposure to mild heat for several days yields vaccine of good potency. In this procedure there is a margin of safety between complete inactivation and appreciable loss of antigenicity. 3. Experience thus far indicates a degree of consistency and reproducibility for the method described. 4. Vaccines made by a combination of ultraviolet irradiation and mild heat appear to retain their antigenicity for at least 7 months.

1. Milzer, A., Levinson, S. O., Shaughnessy, H. J., Janota, M., Vanderboom, K., and Oppenheimer, F., *Am. J. Public Health,* 1954, v44, 26.

2. Wolf, A. M., Shaughnessy, H. J., Church, R. E., Milzer, A., Janota, M., Oppenheimer, F., Morrissey, R. A., Naftulin, H., Chapman, J. W., and Levinson, S. O., *J. A. M. A.,* 1956, v161, 775.

3. Benesi, E., *Gen. Motors Eng.,* 1956, v3, 2.

4. Youngner, J. S., PROC. SOC. EXP. BIOL. AND MED., 1954, v85, 527.

5. Salk, J. E., *Am. J. Hyg.,* 1954, v60, 214.

6. Boyd, T. E., *Bact. Rev.,* 1953, v17, 339.

7. U. S. Public Health Service Minimum Requirements: Poliomyelitis vaccine; *Federal Register,* 1955, v21, 4922.

8. Taylor, A. R., Kay, W. W., McLean, I. W., Jr., Oppenheimer, F., and Stimpert, F. D., *J. Immunol.,* 1957, v78, 45.

Received March 22, 1957. P.S.E.B.M., 1957, v95.

Bruised Tissue. I. Biochemical Changes Resulting from Blunt Injury.* (23185)

MOSTAFA K. HAMDY, FRED E. DEATHERAGE, AND GEORGE Y. SHINOWARA
Departments of Animal Science, Agric. Biochemistry, and Pathology, Ohio State University, Columbus, and Ohio Agric. Exp. Station, Wooster.

A bruise is a tissue injury without laceration usually produced by a blunt object resulting in rupture of the vascular supply and accumulation of blood and fluid. The accumulated blood undergoes, during the healing process, various physical and biochemical changes, represented by the color alteration characteristic of a bruise. Williamson(1) stated that the biochemical studies on wounds and wound healing have been devoted to the metabolism by the injured organism and the determination of the character and metabolism of certain proteins and polysaccharides of the wound area. In this report studies were undertaken to investigate the nature of some of the physical and biochemical changes occurring during the healing process of a bruised tissue.

Materials and methods. Bruising and sampling. Fifty-five cattle were used. The areas to be bruised and symmetrically located control areas were clipped and shaved. Injury was inflicted by 2 blows of a 7 pound sledge hammer over 3 feet in one-half second. (The degree of injury inflicted experimentally was less than that often sustained by cattle due to falls or contacts with other animals and sides of vehicles while in transit.) Observations were made of the bruised area for 9 days after injury. At various intervals animals were slaughtered and the bruised and control areas were excised, sliced, minced, and subjected to various chemical and physical studies. *Easily split iron* (E.S.I.). The tissue sample was homogenized with 0.4% HCl and incubated 24 hours at 37°C(2) and the E.S.I. was determined as the ferrous compound of o-phenanthroline(3). *Pigment extraction.* Saline, buffered to pH 7.4 and con-

* Published with approval of Associate Director, Ohio Agri. Exp. Station. Journal Article No. 60-56. Supported by grant of Farmer Cooperative Service, U. S. D. A. The Rapid Scanning Spectrophotometer, purchased by N.I.H. Grant.

TABLE I. Iron* and Non-Protein Nitrogen Contents of Bruised Cattle Tissue as Related to Gross Appearance and Time.

Age of bruise	Appearance: External — Swelling	External — Color	Fluid — Vol	Fluid — Color	No. of samples	mg/100 g tissue‡: Iron	mg/100 g tissue‡: N.P.N.
Control†	—	Normal	—	Normal	30	.25 ± .07	650 ± 18
15 min.	++	Dark red	++	Red	4	.30 ± .03	560 ± 20
2 days	+++	*Idem*	+++	Dark red	4	.32 ± .04	460 ± 15
3	++	Light green-purple	++	Brown-yellow	5	.46 ± .05	410 ± 13
4	+	Yellow-green-purple	+	Yellow-orange	3	.50 ± .04	320 ± 12
5	—	Orange	±	*Idem*	5	.57 ± .05	140 ± 8
6	—	Slight orange	±	Slight yellow	3	.32 ± .04	200 ± 12
7	—	Normal	—	—	3	.25 ± .06	240 ± 10
8	—	"	—	—	2	.27 ± .05	500 ± 18
9	—	"	—	—	2	.32 ± .03	720 ± 16

* "Easily split iron."
† Control (normal) tissues from symmetrically located areas as the bruises.
‡ Mean ± S.D.

taining 1% normal human serum albumin, was used for extraction of some of the pigments present in the bruised and control tissues, followed by chloroform extraction. *Hemoglobin and bilirubin.* The American Optical Rapid Scanning Spectrophotometer was used for qualitative studies of these pigments in saline and chloroform extracts. The concentrations of hemoglobin and bilirubin in the saline extract were determined(4) using a Beckman D.U. spectrophotometer. Bilirubin concentration in the chloroform extract was determined spectrophotometrically at 450 mμ. *Non-protein nitrogen (N.P.N.).* A modification of the Miller and Miller(5) procedure was used for determination of the N.P.N. in the saline extract.

Results. In Table I are recorded observations of bruises inflicted on cattle using the technic described. It is evident that the mean concentration of iron (E.S.I.) in bruised tissue rose immediately up to and over the control level (0.25 mg %) in 4-5 days after injury and then decreased to reach the control level within 7-9 days, at which time the bruise had apparently healed. The non-protein nitrogen, on the other hand, decreased from the control level of 650 mg% to 140 mg% in 5 days, but rose abruptly to the control value in 9 days.

Of the total pigment in exsanguinated normal muscle tissue, myoglobin is quantitatively the most important(6), whereas hemoglobin represents only approximately 10% (7,8). Therefore, the data given in this paper for normal (control) tissue are for both myoglobin and hemoglobin. Fig. 1 represents typical spectrophotometric absorption curves of saline extracts of normal and of 5-day-old bruised tissue samples. Both extracts differ in several ways. The greater absorption at 560 and 575 mμ (corrected for absorption curve of the didymium glass standard) of bruised tissue extract than that of normal tissue, is attributed to increase in hemoglobin concentration. The plateau at 600-640 mμ in bruised tissue extract suggests the presence of alkaline hematin which is absent in the control extract. The absorption spectrum of a pure bovine hemoglobin is shown in Fig. 2 for comparative purposes.

After the presence of hemoglobin in the bruised tissue was established it was decided to follow quantitatively its changes during the healing process. The results (Table II) indicate that extra-stromal hemoglobin concentration increased immediately following bruise infliction, followed by a decrease to reach the level of normal tissue upon complete healing. It appears therefore that any increase in red pigment in bruised tissue compared to that of the control, is due primarily to hemoglobin and traces of hematin. Apparently there is very little change in myoglobin concentration due to bruising.

The concentration of the yellow pigments,

TABLE II. Relation between Extra-Stromal Hemoglobin and Bilirubin Concentrations in Bruised Cattle Tissue.

	Concentration in mg/100 g tissue					
	Myoglobin and hemoglobin		Yellow pigments as bilirubin			
			Total		Bilirubin	
Age of bruise	Control	Bruised	Control	Bruised	Control	Bruised
15 min.	52.5	180.2	.76	.43	.00	.00
2 days	42.4	228.9	.82	2.28	"	1.40
3	40.6	425.2	.45	4.17	"	3.52
4	60.2	500.2	.86	4.30	"	3.89
5	57.5	372.0	.67	2.36	"	1.90
7	71.8	390.6	.32	1.80	"	1.39
8	78.2	80.5	.50	2.20	"	1.30
9	60.2	68.2	.40	.50	"	.20

representing the total of that found in both the saline and chloroform extracts, was then determined spectrophotometrically with C. P. bilirubin (Eastman) as a standard. The results obtained on control and bruised tissues are recorded in Table II. It can be seen that total yellow pigments in normal tissue ranged from 0.32 to 0.86 mg/100 g, while that of bruised tissues increased to 4.3 within 4 days of injury, followed by a rapid decrease, to reach the normal tissue level. Total bilirubin was then determined chemically by the van den Bergh reaction on the saline and chloroform extracts of control and bruised tissue: The results are shown in Table II. It is evident that control tissue extract did not contain bilirubin, *per se*, while its concentration in bruised tissue extracts rose to 3.8 mg/100 g tissue within 4 days after injury and then decreased to 0.20 mg/100 g by the ninth day. Therefore, the increase in total yellow pigments due to injury is attributed to bilirubin. This was confirmed by isolating the pigment as the barium salt: The free pigment had a maximum absorption at 450 mμ, identical to that of C. P. bilirubin. The yellow pigments in the normal (control) tissue must be substances other than bilirubin such as lipochromes, riboflavin and hemoglobin, all of which absorb light at 450 mμ.

Discussion. The increase of extra-stromal hemoglobin during early stages of healing of bruised tissue apparently resulted from destruction of red blood cells which have escaped from the circulation upon rupture of the capillaries. This increase of extra-stromal hemoglobin occurred along with an increase in iron (E.S.I.) content of bruised tis-

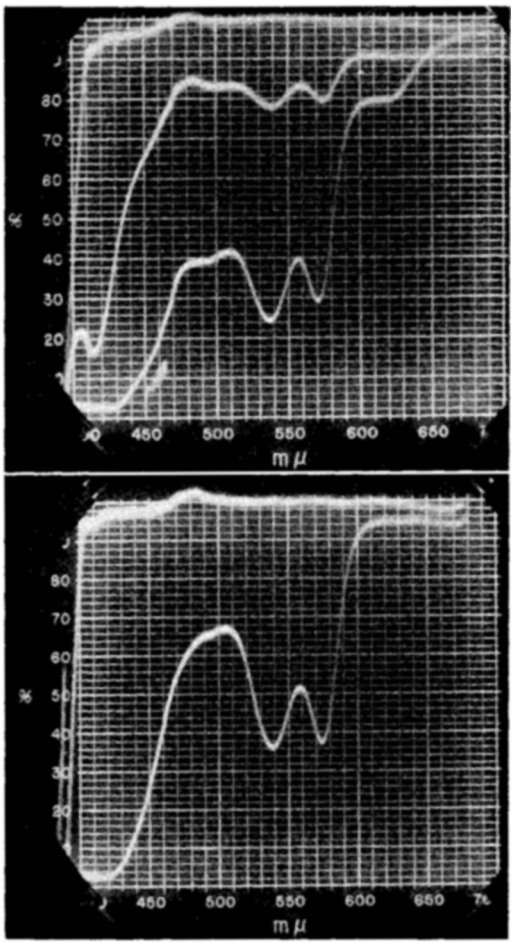

FIG. 1 (top). Spectrophotometric absorption curves of saline extracts of a 5-day-old bruise in cattle and a control (non-bruised) tissue from a symmetrically located area, first (top) curve, saline reference; second curve, control extract; third (bottom) curve, bruise extract.

FIG. 2 (bottom). Spectrophotometric absorption curves of bovine hemoglobin. First curve, saline reference; second curve, hemoglobin solution, approximately 70 mg/100 ml saline.

sue, indicating that this hemoglobin was catabolised in the tissue by release of its iron. No explanation is given for the drop of the N.P.N. in early stages of healing, but the higher concentration of this N.P.N. after 4-5 days may be due to excessive tissue catabolism and degradation of the globin moiety of the hemoglobin molecule. Selye and Dosne (9) found that blood which passed through a damaged area contained a high concentration of free hemoglobin and N.P.N. The absence of bilirubin immediately after bruising the tissue and its rapid increase in 3 or 4 days followed by rapid decrease in concentration seem to indicate that this pigment is a degradation product from the catabolism of free hemoglobin. It should be stated that increase of bilirubin concentration coincided with increase of the orange color usually observed during the healing process of a bruise. The detection of hematin (Fig. 1) in the bruised tissue during healing seems to indicate that globin is first split off from the hemoglobin molecule giving rise to hematin. The latter loses its iron and appears to undergo oxidation resulting in the opening of the tetrapyrrolic ring of the molecule in the α-position with loss of α-methyne bridge (–CH =) and formation of 2 hydroxyl groups at the end of the linear tetrapyrrolic molecule. It is possible that biliverdin is the first compound formed which may be reduced by the reducing system of the tissues to form bilirubin as indicated by Lemberg and Wyndham(10). These physical and biochemical changes during healing of bruised tissues were also investigated in 100 rabbits and the results followed the same pattern. The sequence of events resulting in the degradation of hemoglobin during healing of a bruised tissue is consistent with the data of *in vitro* experiments reported by Lemberg and Legge(11).

Summary. In experimentally inflicted bruises of cattle, the greatest swelling and fluid volume occurred within 2 days. The maximum biochemical changes in bruised tissue were found on or about the fourth or fifth day: These included a drop in N.P.N. to one-fourth of the control level and a 2-fold increase in the "easily split iron" concentration. Moreover, the 10-fold elevation in red pigment was due to extra-stromal hemoglobin. Bilirubin formation in bruised tissue has been demonstrated conclusively and the probable metabolic derivation of this pigment is discussed. Although healing was evident grossly in 7 days, the biochemical values did not return completely to the control levels until the ninth day.

1. Williamson, B. M., *Clin. Chem.,* 1956, v2, 1.

2. Barkan, G., and Walker, B. S., *J. Biol. Chem.,* 1939, v131, 447

3. Fortune, W. B., and Mellon, M. G., *Ind. and Eng. Chem. Anal. Ed.,* 1938, v10, 60.

4. Shinowara, G. Y., *Am. J. Clin. Path.,* 1954, v24, 696.

5. Miller, G. L., and Miller, E. E., *Anal. Chem.,* 1948, v20, 481.

6. Biorck, G., *Acta med. Scandinav. suppl.,* 1949, v226, 222.

7. Wierbicki, E., Cahil, V. R., Kunkle, L. E., Klosterman, E. W., and Deatherage, F. E., *Agr. and Food Chem.,* 1955, v3, 244.

8. Shenk, J. H., Hall, J. L., and King, H. H., *J. Biol. Chem.,* 1934, v105, 741.

9. Selye, H., and Dosne, C., PROC. SOC. EXP. BIOL. AND MED., 1941, v47, 145.

10. Lemberg, R., and Wyndham, R. A., *Biochem. J.,* 1936, v30, 1170.

11. Lemberg, R., and Legge, J. W., Hematin compounds and bile pigments, Interscience Publishers, N. Y., 1949.

Received March 22, 1957. P.S.E.B.M., 1957, v95.