the dog and the rat following RIAP administration indicates that the liver metabolizes RIAP to at least four I^{131}-containing compounds, one of which is free I^{131} itself. It is of interest that approximately 95% of the radioactivity excreted in the bile is in the form of 3 as yet unknown compounds. Chaikoff(7) has shown that rat liver is rich in a "nonspecific deiodinase," capable of splitting the I^{131} tag from various thyroid hormone intermediates. It is possible that such a deiodinase is also responsible for splitting the I^{131} tracer from RIAP.

The major radioactive metabolic products found in the urine of the dog and the rat appear to be identical to those found in the bile following RIAP administration. Whether or not these represent metabolic products of the liver which are simply excreted by the kidney, or whether they are metabolites of the kidney itself is not known. The unknown radioactive metabolites (X, Y and Z in Fig. 3) of RIAP have not been demonstrated in the plasma.

Summary and conclusions. These studies show that the dog, like the rat, rapidly converts RIAP to a more diffusible form and that the liver is the primary site of this conversion. Chromatographic analysis of serial plasma samples following RIAP injection in the rat indicated that as early as 15 minutes most of the plasma radioactivity was due to free I^{131} and not RIAP, thus invalidating the use of this compound in total body water studies. Finally, chromatographic analyses of dog and rat bile following RIAP administration showed that in addition to RIAP and free I^{131}, the majority of radioactivity excreted was in the form of 3 as yet unknown compounds.

1. Talso, P. J., Lahr, T. N., Spafford, N., Ferenzi, G., Jackson, H. R. O., *J. Lab. and Clin. Med.*, 1955, v46, 619.

2. Sapirstein, L. A., Hanusek, G. E., *Am. J. Physiol.*, 1958, v193, 272.

3. Krasnow, N., Levine, H. J., Wagman, R. J., Gorlin, R., *Circ. Res.*, 1963, v12, 58.

4. Sullivan, J. M., Rose, J. C., *J. Lab. and Clin. Med.*, 1961, v57, 955.

5. Sullivan, J. M., Straub, W. H., Rose, J. C., PROC. SOC. EXP. BIOL. AND MED., 1961, v108, 686.

6. Russell, J. A., *Am. J. Physiol.*, 1942, v136, 95.

7. Tong, W., Taurog, A., Chaikoff, I. L., *J. Biol. Chem.*, 1954, v207, 59.

8. Straub, W. H., Sullivan, J. M., Rose, J. C., *Georgetown Med. Bull.*, 1962, v16, 20.

Received May 6, 1964. P.S.E.B.M., 1964, v116.

Decrease in Incidence of Blood Spots in Chicken Eggs by Injection Derivatives of Pyrrole-2-Aldehyde Chalcones into Hens.* (29469)

C. H. BIGLAND†, ESTHER B. BENNETT AND URSULA K. ABBOTT

School of Veterinary Medicine and Department of Poultry Husbandry, University of California, Davis

The cause of preovulatory, intrafollicular hemorrhage, resulting in blood spots in chicken eggs has not been determined; however, it is thought to be related to capillary fragility(1). Flavone glucosides and their water-soluble derivatives, the chalcones, have been used experimentally in the treatment of disease of man in which capillary fragility is a prominent finding(2,3). Therefore, the effect of 4 newly synthesized pyrrole-2-aldehyde chalcones on the incidence of blood spots in eggs was tested and compared with that of commercial hesperidin and hesperidin methyl chalcone.

Methods. Laying Leghorn chickens of a strain which produces a high percentage (50 to 100%) of eggs with blood spots,‡ were injected with the test materials. The pyrrole-

* This work was supported by NIH Grant HE 08288-01.

† Present address: Coll. of Vet. Med., Univ. of Saskatchewan, Saskatoon, Canada.

‡ Developed by Dr. F. W. Lorenz, University of California, Davis, Calif.

2-aldehyde chalcones were prepared by modified standard base condensation methods(4), using pyrrole-2-carboxaldehyde and its N-methyl derivative, together with various methyl-phenyl ketone derivatives. Solutions (2-2.5 ml) containing chalcones were injected subcutaneously into groups of laying chickens daily as follows:

Group 1. hesperidin, 10 mg;
Group 2. hesperidin methyl chalcone, 25 mg;
Group 4. N-methyl-pyrrole-2-al-(3′-hydroxy) acetophenone, 150 mg;
Group 5. N-methyl-pyrrole-2-al-(3′-amino-4′-methoxy) acetophenone, 100 mg;
Group 7. N-methyl-pyrrole-2-al-(2′, 3′, 4′-trihydroxy) acetophenone, 100 mg;
Group 8. N-methyl-pyrrole-2-al-(4′-hydroxy-3′-methoxy) acetophenone, 50 mg.

Injections were given for 7 days in Groups 1 and 2, and 14 days in Groups 4, 5, 7, and 8. Groups 3, 6, and 9 were non-injected controls.

From 2 days following the first injection to 2 days after the last injection was considered the experimental period. This procedure avoided the inclusion of ova already preformed at the time of initial injection. Eggs laid 8 days before and 8 days after this period were considered pre- and postinjection levels.

Experimental birds were selected from a high blood-spot-producing strain of Leghorn chickens which had exhibited an increasing incidence of blood spots for 5 months prior to injection. Only birds which laid consistently throughout the test periods were chosen for tabulation, in order to avoid sampling errors. Lee-White whole blood clotting times, one-stage prothrombin times, and thromboplastin whole blood clotting times were determined. Also, measurements of hematocrit values, buffy coat, and plasma volume, as well as red blood cell counts and white cell differential counts, were made.

Although the size and number of blood spots were graded on egg break-out according to a previously prepared formula, only the presence or absence of blood spots was used for tabulation and statistical analysis (9).

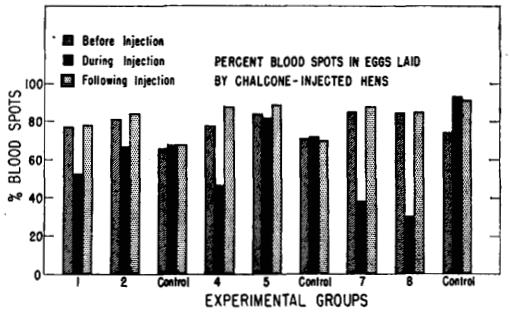

FIG. 1

Results. In 3 groups the percentage of eggs with blood spots was lowered significantly ($p = .001$) from pre-injection levels by the injection of chalcones (Fig. 1). In Group 4 the reduction was from 78% to 47%; in Group 7, from 85% to 38%; and in Group 8, from 85% to 30%. The percentages of blood spots in these groups were also significantly lower than those of their non-injected controls. Eggs from birds in Groups 2 and 5 failed to show statistically significant decreases in blood spot percentage. However, in Group 1, blood spot incidence was decreased from 77% to 52%, but this reduction was only statistically significant ($p = .02$-$.01$) when compared with before and after injection levels, not with non-injected controls. It was observed that the number and size of blood spots also were reduced, by the effective chalcone injections, in eggs which could not be recorded as negative. No significant changes due to the injections were noted in blood coagulation tests or hemograms. No generalized toxic reactions were evident as a result of the injections; all birds continued to eat, drink, and lay in a normal manner. Some local swelling, thought to be due to impurities, occurred at site of injection in Groups 1, 2 and 7. Rate of reduction in blood spot incidence was generally rapid 2 days after injection of the effective chalcones. This was followed by a quick return to high blood-spot percentages, 2 to 4 days after cessation of injections (Fig. 2).

Discussion. The significant reduction in incidence of blood spots following injection of chalcones indicates that the cause of the

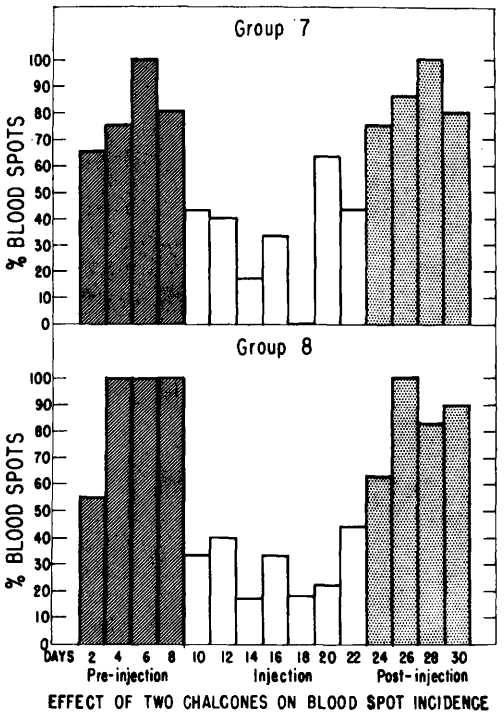

FIG. 2

intrafollicular hemorrhage resulting in blood spots in chicken eggs is due to capillary fragility. In this experiment, this condition was corrected, at least in part, by 3, and possibly 4, of the chalcones injected. If the chalcones possess anti-hyaluronidase activity, such as is reported for phosphorylated hesperidin(5,6), their mode of action may be reduction of effect of this enzyme in the ovary (7).

The chalcones used in this experiment are believed to be the first products known that consistently reduce incidence of blood spots. This reduction was accomplished in a line of chickens that normally produces a high percentage of eggs containing blood spots. However, inconsistent decreases in percentage incidence of blood spots previously have been recorded with birds fed high levels of "cerograss," or which had access to free range(8).

The quick and specific action of 3 chalcones in reducing incidence of blood spots indicates that chickens of the line tested may be useful experimental animals because of a natural ovarian capillary fragility. Such birds may be used in assaying effectiveness of new drugs or procedures for treatment of capillary fragility and hemorrhage. Also, they may be useful in studying the pharmacologic action of flavones and chalcones, and in determining causes of capillary fragility.

The rapid response to injections of the chalcone derivatives is in contrast to the slow response noted with rutin or hesperidin in treatment of vascular purpura in man. In our experiments the dosage per bird was approximately 25 to 50 times larger than the comparative dose, based on body weight, of 300 mg of rutin and 150 mg of hesperidin used with human patients(2).

Summary. Six types of chalcones were tested for effect on incidence of blood spots in chicken eggs. Single daily subcutaneous injections of 3 pyrrole-2-aldehyde chalcones significantly reduced, from pre-injection levels, the percentage of blood spots in eggs laid by a high blood-spot-producing line of Single Comb White Leghorns, from 78 to 47, 85 to 38 and 85 to 30, respectively, during 14 days of injection. No significant effect of chalcone injections on whole blood clotting time, prothrombin time, hematocrit values, buffy coat, erythrocyte count, or differential white blood cell counts was noted. No systemic toxic reaction was observed in chickens injected with the chalcones. A slight local reaction believed due to impurities was noted with 3 of the 6 chalcones used. Since certain chalcones are known to reduce capillary fragility in man, it is assumed that the problem of blood spots in eggs probably is related to capillary fragility.

The technical assistance and synthesis of the new chalcones by Mr. A. Hamidi is acknowledged.

1. Nalbandov, A. V., Card, L. E., *Poultry Sci.*, 1944, v23, 170.

2. Stefanini, M., Dameshek, Wm., *The Hemorrhagic Disorders*, Grune & Stratton, N. Y., 1962.

3. Yokotani, Hajime, *Nippon Takurigaku Zask*, 1960, v56, 369.

4. Hamidi, A., *Chalcone Derivatives from Pyrrole-2-aldehyde*. Thesis. Univ. of The Pacific, Stockton, Calif., 1963.

5. Beiler, J. M., Martin, G. J., *J. Biol. Chem.*, 1948, v174, 31.

6. Cohen, H., Freedman, H. H., Kleinberg, Wm.,

Eisler, M., Martin, G. J., PROC. SOC. EXP. BIOL. AND MED., 1953, v82, 749.

7. Kurzrok, R., *Ann. N. Y. Acad. Sci.*, 1950, v52, 1180.

8. Nalbandov, A. V., Card, L. E., *Poultry Sci.*, 1947, v26, 400.

9. Snedecor, G. W., Chap. 9, *Statistical Methods*, 4th ed., Iowa State Coll. Press, Ames, 1946.

Received May 11, 1964. P.S.E.B.M., 1964, v116.

Aortal Mucopolysaccharide Changes After Epinephrine Administration in Rabbits. (29470)

L. J. MILCH AND P. B. LOXTERMAN
Miles Laboratories, Inc., Elkhart, Ind.

Lorenzen(1) reported gross aortic lesions involving medial necrosis and occasional intimal proliferation after 15 days of intravenous epinephrine in rabbits. At the same time, based on hexosamine measurements and assay of the S^{35} content of dried tissue, Lorenzen suggested that the pathosis observed was associated with an accumulation of acid mucopolysaccharide (MPS) and that this accumulation is probably a reflection of reparative processes started by the epinephrine-induced injury.

Lorenzen's data are subject to additional interpretation in that his results also support the hypothesis that epinephrine initiates and aggravates a defect in the "biochemical architecture" of aortal wall which renders it susceptible to degenerative change, and that the defect is manifested by an alteration in aortal MPS metabolism. Complete proof of such a hypothesis would involve rather extensive experimentation, but an important segment of that proof requires that intravenous epinephrine will induce aortal MPS changes prior to, and in the absence of, gross pathosis. Accordingly, it was decided to administer epinephrine to rabbits over a portion of the dosage schedule described by Lorenzen and determine if alterations of MPS metabolism can be identified in the wall of the aorta.

Methods. Twenty-two male and female rabbits of the New Zealand strain (avg wt 4.5 kg) maintained on Rabbit Chow and water *ad lib* were housed in individual cages and divided equally into 2 groups. Group E animals were injected (ear vein) twice daily for 5 days with 0.025 mg/kg of epinephrine in physiological saline. Group C received the saline alone in equivalent volumes on the same schedule. On the evening of the fifth day, each rabbit was given 300 μc of carrier-free $Na_2S^{35}O_4$ in physiological saline by subcutaneous injection and all animals were sacrificed 16 hours later. Examination of aortae at autopsy failed to identify gross pathosis of any sort with the exception of a single intimal lesion in the thoracic aorta of C-5. Aortae were excised, cleaned of adhering fatty tissue and individually digested in a papain EDTA-cysteine-Po_4 buffer mixture (pH 5.5) at 60° for 64 hours. NaOH was added to each solubilized tissue sample to a final concentration of 0.5 N and agitated gently at 4°C for 4 hours. The pH was then adjusted to 5.5 with 5 N HCl and MPS were isolated and reprecipitated to constant S^{35} specific activity by successive separations in 75% ethanol in water. The final precipitate was taken up in 0.25 ml of water and this solution assayed for MPS content(2)* and MPS specific activity.†‡ Electrophoresis of this solution on cellulose acetate strips (formic acid-pyridine buffer, pH 3) demonstrated 2 acridine orange positive components differing only slightly in mobility. The faster of the

* Repeated recovery studies of added glucuronolactone to MPS isolated from epinephrine and control rabbit aortae ranged from 100-110%.

† When 10^4 inorganic S^{35} counts were added to enzymatic digests of non-radioactive rabbit aortae, and the MPS isolated by 3 successive precipitations with 75% ethanol in water, the final precipitates displayed background levels of radioactivity.

‡ Scintillation Counter, Packard Model 314EX.