LH-releasing factor, acts directly on the adenohypophysis.

The residual OAAD produced by SME extract in the hypox. rat is presumably due to the presence of LH or vasopressin or a combination of the 2 in the extracts.

Since the difference between the OAAD in normal and hypophysectomized assay rats injected with SME extract was not large, it would appear that the extracts caused release of only a small amount of LH from the hypophysis. As we have shown that content of LH in the pituitary of these immature assay animals is very low, the amount of LH released by the extract is probably a very significant percentage of this total. Their low hypophysial LH content may constitute a major disadvantage to use of these immature rats for assay of LH-releasing activity. Nevertheless, the data show that a significant release of LH was produced by SME extracts in these assay animals.

Summary. Acid extracts of rat SME tissue evoked OAAD in immature rats pre-treated with gonadotrophins. Part of the activity in the extracts could be accounted for by their content of LH or vasopressin or both, whereas the remaining activity appeared to be due to release of LH from pituitary of assay rats. The substance(s) responsible for LH-releasing activity of extracts has been called LH-releasing factor. The nature of this material is unknown, but it appears to differ from histamine, serotonin, substance P, epinephrine, and vasopressin or oxytocin.

The technical assistance of Maria Smith is gratefully acknowledged.

1. Harris, G. W., *The Neural Control of the Pituitary Gland,* Arnold, London, 1955.

2. McCann, S. M., Taleisnik, S., Friedman, H. M., *Fed. Proc.,* 1960, v19, 292.

3. Saffran, M., Schally, A. V., Benfey, B. G., *Endocrinol.,* 1955, v57, 439.

4. Guillemin, R., Hearn, W. R., Cheek, W. R., Householder, D. E., *ibid.,* 1957, v60, 488.

5. Royce, P. C., Sayers, G., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 1.

6. McCann, S. M., Haberland, P., *ibid.,* 1959, v102, 319.

7. Parlow, A. F., *Fed. Proc.,* 1958, v17, 402.

8. Mindlin, R. L., Butler, A. W., *J. Biol. Chem.,* 1937, v122, 673.

9. Harris, G. W., Jacobsohn, D., Kahlson, G., *Ciba Foundation Colloquia (Endocrinology IV),* 1952, 186.

10. Udenfriend, S., Shore, P. A., Bogdanski, D. F., Weissback, H., Brodie, B. B., *Recent Prog. Hormone Research,* 1957, v13, 1.

11. Van Dyke, H. B., Adamsons, K., Engel, S. L., in *The Neurohypophysis,* ed. Heller, H., 1957, 67.

12. Martini, L., Mira, L., Pecile, A., Saito, S., *J. Endocrin.,* 1959, v18, 245.

Received May 13, 1960. P.S.E.B.M., 1960, v104.

Crotalaria spectabilis Toxicity in Chickens.* (25865)

J. R. ALLEN, G. R. CHILDS† AND W. W. CRAVENS†

Dept. of Pathology, University of Wisconsin School of Medicine, Madison

Crotalaria spectabilis, commonly known as rattlebox, is a leguminous plant grown throughout the South as a cover crop on sandy soils. It grows wild along fence rows, in uncultivated fields and occasionally in row crops after cultivation has been completed. The plant, as well as its seed, is toxic to all farm animals. The toxic principle in *Crotalaria spectabilis* has been isolated and identified as monocrotaline(1,2). Neither lethal dose nor tissue changes associated with its consumption have been clearly defined in chickens(7). Toxicity studies of *Crotalaria spectabilis* for poultry were undertaken after numerous deaths in several large laying flocks that consumed a ration containing an unknown quantity of this legume. Examination of dead and morbid birds invariably revealed ascites and hemorrhage in the liver. It was

* This investigation was supported in part by a grant from Nat. Heart Inst., P.H.S.

† McMillen Feed Mills (Research), Decatur, Ind.

TABLE I. Response of Chicks to Different Concentrations of Crotalaria in the Diet.

Conc. of Crotalaria in diet (%)	No. of chicks that died	Time interval for death (days)	Wt at 5 wk (g)
.0	0/10		596
.05	"		443
.1	2/10*	23-28	312
.2	3/10	18-32	211
.3	9/10	15-35	178
.0	0/10		
.5	10/10	7-18	
1.0	"	5-11	
3.0	"	5-8	
5.0	"	4-7	

* 2/10 signifies that 2 out of 10 birds died within specified interval.

also common to find ruptured liver with hemorrhage into the abdominal cavity of such chickens.

Methods. Fifty Vantress-White Rock sexed broiler chicks were weighed at one day of age and placed on rations containing from 0.5% to 5.0% *Crotalaris spectabilis* seed in a complete commercially prepared broiler ration.† All the test birds died within 19 days. In a second study the level of *Crotalaria spectabilis* was reduced to levels ranging from 0.05% to 0.3%. Surviving birds in the second trial were weighed again at 5 weeks of age. Response of the birds to different concentrations of *Crotalaria spectabilis* in various assays is shown in Table I. Autopsies were performed on all birds that died as well as survivors. Tissue from the heart, liver, kidney, spleen and bone marrow was fixed in 10% formalin, embedded in paraffin and sections cut at 6 μ and stained with hematoxylin and eosin.

Pathology. All levels of *Crotalaria spectabilis* seed produced reduction in growth. As the level was increased, growth retardation was more evident. At concentrations in excess of 0.3% all birds died. Ascites was present in all birds except those in the 2 control rations. Ascitic fluid varied directly with amount of seed fed. Thirty to 75 ml of peritoneal transudate was frequently aspirated from 3 to 5 week old birds. Total protein in this transudate ranged from 0.8 to 2.4 g %. A marked reduction in size of the liver was a constant finding irrespective of concentration of Crotalaria fed. Birds receiving higher concentrations developed these lesions within a shorter period of time. It was not uncommon to find livers less than 1/3 the size of control birds (Fig. 1). These light tan livers were frequently covered by coagulated transudate. Occasionally in chickens fed lower levels (0.1-0.2%) there was a rupture of the liver capsule with hemorrhage into the abdominal cavity. There was also minimal fibrous hyperplasia of the liver capsule. It was not uncommon to find petechial or ecchymotic hemorrhages in liver and lungs. Focal hemorrhages were also seen in the pericardium of some birds. Bone marrow in the test birds was normal.

Discussion. Initially the causative factor responsible for numerous deaths in several large laying flocks was not known. Tentative considerations were toxic fat, excessive salt in the ration and creosote toxicity resulting from disinfectants. These were excluded on the basis of feeding trials and chemical analy-

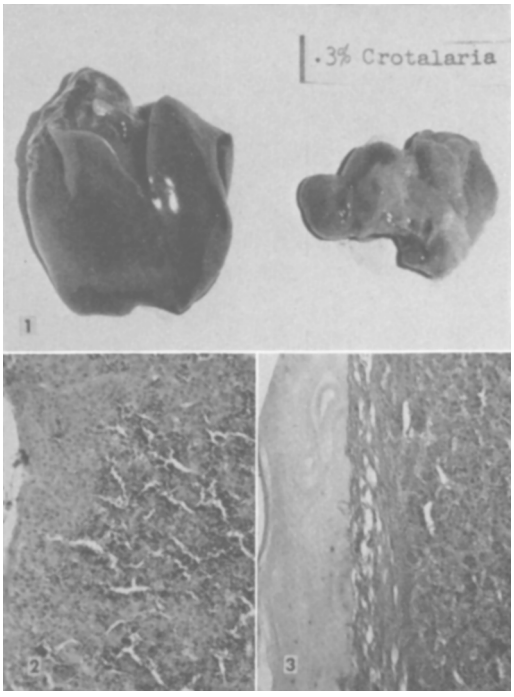

FIG. 1. Differences in size of liver of bird fed 0.3% Crotalaria when compared to control.
FIG. 2. Hemorrhage within liver parenchyma. H & E stain; ×75.
FIG. 3. Coagulated transudate over liver capsule. H & E stain; ×75.

sis of the feed. Inspection of the feed revealed seeds resembling *Crotalaria spectabilis*. Thereafter, inclusion of such seeds in test rations produced lesions similar to those observed in the field.

Crotalaria depresses egg production and causes weight loss in laying birds. Crotalaria is known to produce firm and cirrhotic livers in horses(3,4,5). In chickens that died in these acute toxicity studies cirrhosis was not observed. Cattle consuming Crotalaria seed or plant have a loss of appetite, tenesmus and blood in the feces. Pigs eating Crotalaria develop hemorrhage into the serous membranes, anemia, enteritis, and jaundice(6). No anemia, enteritis, or jaundice was observed in the chickens in these assays.

Summary. Concentrations of 0.05% to 5% *Crotalaria spectabilis* seed were fed to chicks. All concentrations of the legume were shown to be toxic. Concentrations in excess of 0.3% produced death in all birds within 18 days. When less than 0.2% was fed there was a marked reduction in weight gain. In birds which died, hemorrhage was frequently observed in liver, lungs and the pericardium. Atrophy of liver and ascites were constantly encountered in test birds after death.

1. Adams, R., Rogers, E. F., *J. Am. Chem. Soc.,* 1939, v61, 2815.

2. Neal, W. M., Rusoff, L. L., Ahmann, C. F., *ibid.,* 1935, v57, 2560.

3. Gibbons, W. J., Durr, E. H., Cox, S. A., *North Am. Vet.,* 1953, v34, 556.

4. Piercy, P. L., Rusoff, L. L., *J.A.V.M.A.,* 1946, v108, 69.

5. Ritchey, G. E., McKee, R., Becker, R. B., Neal, W. M., Arnold, P. T., *Univ. of Florida Agri. Exp. Station Bull.,* 1941, 361.

6. Berry, D. M., Bras, G., *North Am. Vet.,* 1957, v38, 323.

7. Schmittle, S. C., Richey, D. J., Tumlin, J. T., 48th Meeting Poultry Science Assn., Ames, Iowa, Aug. 1959.

Received May 16, 1960. P.S.E.B.M., 1960, v104.

Tolerance of Chickens for Barium. (25866)

DEWEY JOHNSON, JR., ARNON L. MEHRING, JR., AND HARRY W. TITUS
Limestone Products Corp. of America, Newton, N. J.

Barium carbonate has been used for poisoning rats for many years. Several terms have been used in the literature to describe levels of barium that are toxic to animals. Lethal dose and fatal dose have been used in European literature, and LD_{50} dose in recent American literature. Hüter(1) considered the lethal dose of barium carbonate for rats to be 1500 mg/kg of live weight. Dieke(2) found that LD_{50} of barium carbonate for wild rats was 1480 mg/kg. The LD_{50} of barium, based on Dieke's observations would be about 1030 mg/kg. According to Dervilleé and Raveleau(3), the range in fatal dose of barium, given in the form of carbonate, is 35-56 mg, 104-139 mg, and 418-557 mg, respectively, for the cat, guinea pig, and rabbit, per kilo of live weight. In view of wide differences among tolerances of different mammalian species for barium, it was of interest to study the tolerance of the chicken. Barium hydroxide and barium acetate were chosen for this study because they are among the most soluble of all barium compounds.

Procedure. Two types of experiments, single-dose toxicity and growth, were used. Single, graded doses of barium as barium hydroxide were administered to 40 male chickens 7 weeks old with average weight of 943 $\pm$ 44 g. The required quantities of barium hydroxide to supply 400, 500, 600, 700, and 800 mg of barium were weighed to nearest mg and packed into gelatin capsules. A capsule was placed as far down each chicken's esophagus as possible; then, by stroking the chicken's neck, the capsule was moved into the crop. Procedures in the two growth experiments were similar to those described by Mehring, *et al.*(4). In Exp. 1, 12 lots of chicks (10 males and 10 females per lot) were