A Hemorrhagic and Rachitic-Like Syndrome in Chickens Due to Nitrofural[1] Medicated Feed

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INTRODUCTION

In young birds there are many hemorrhagic conditions with poorly understood mechanisms. In addition to hemorrhages associated with infectious diseases (fowl-pest, Newcastle disease, fowl-cholera, septicemia, blue-comb disease . . .), parasites (coccidiosis) and intoxication [heavy metals, fungi, soybean oil meal extracted with trichloroethylene (Eveleth and Goldsby, 1953) and underheated soybean oil meal, (Balloun and Johnson, 1953)], there are two types of hemorrhages having a non-precise etiology. The first is characterised by profuse hemorrhages with considerable increase in the blood clotting time and "quick" time test (Goldhaft and Wernicoff, 1954; Cover *et al.*, 1955). This condition is considered as being due to a vitamin K deficiency. The second is a syndrome characterised by numerous, small, localised hemorrhagic lesions, normal prothrombin-time (Wartell, 1957), and hemogram modifications due to reticulohistiocytary lesions. Until now, the cause of this condition has been unknown. Observation of cases of hemorrhagic diathesis of this second category seems to indicate that certain of them should be attributed to a particular type of nitrofural intoxication.

OBSERVATIONS

The condition developed in several flocks of chickens of from 3 to 6 weeks old, being fed with commercial feeds containing cereals, wheat bran, meat and fish meal, alfalfa flour, analyzing about 6% crude fibre, 13% mineral salts, over 18% protein, 3% fat, and high proportions of vitamins A, D_3 and the B complex.* In certain cases an antibiotic was added to the ration. All the birds that developed the condition were being given 0.05 to 0.2 gram of nitrofural per kilogram of feed.

In the majority of cases the symptoms appeared abruptly. These were anemia, asthenia and anorexia, causing the death of a number of subjects, in from a few hours to several days. Some of them showed diarrhea. Limping was frequent, and in one flock, the majority of the sick birds were unable to walk. The birds that did not die remained weak, anemic and thin, and often developed rapidly evolving skeletal deformities. This osteopathia is similar in clinical allure to an intense rickets condition. These birds, when given a different diet without nitrofural, can regain apparently normal health, except for the irreversible bone deformities, and the considerable delay of growth.

In cases of rapid death, the macroscopic lesions are very characteristic of a hemorrhagic syndrome. The mucosae are pale and there are numerous small bloody suffusions scattered throughout the subcutaneous or intermuscular conjunctive

[1] Nitrofural is the common French name for nitro-5-furaldehyd-2-semi-carbazone, generally sold under trades names such as: "Nitrofurazone, Furacine."

* The approximate content (per kg.) of these feeds being: Vit. A 3–5000 I.U., Vit. D_3 500–1000 I.U., Vit. B_1 2 mg., Vit. B_2 3.2–3.5 mg., Pantothenic acid>10 mg., Vit. PP>18 mg., Biotin>0.5 mg., Vit. B_{12} 10–20 mcg.

tissue and in the thymic nodules of the neck. There are petechiae and suffusions on the heart and the intestinal mucosa, and hemorrhages in the marrow of long bones, especially in the growth zones of these bones.

In cases where the progress of the disease was slower, there is an anemia and thinness, noticeable immediately upon opening the dead bird. Also, there is a pericardial transudate, marked duodenal congestion, myocarditis, hepatic or renal degeneration, and especially numerous hemorrhages clotting in the hollow organs and spots of blood being resorbed throughout the connective tissue. A slight icterus can exist.

In one flock, the majority of affected birds had intense skeletal lesions characterized by a generalized malacic condition, with enlargements in the articular regions of long bones and rotation of the feet. A longitudinal section of a long bone, such as the tibia, showed a normal articular cartilage; a slightly wavy, irregularly thickened epiphysial cartilage; and osteoporosis and enlargement of the epiphysis. Sub-periosteal and intra-osseous hemorrhages were present in the softened epiphysis. The distal parts of the diaphyses were fragile, due to an almost complete absence of bone cortex, and a median section of the bone, although appearing normal, showed exaggerated flexibility. The diaphyseal marrow had a fatty-white color, unlike that of the epiphyseal regions.

X-rays confirmed the generalized osteoporosis, and the almost complete disappearance of calcified tissue in the epiphyses, with persistence of epiphyseal cartilage that is either normal, or very slightly thickened, in isolated spots.

DISCUSSION

The etiological study was based on a series of epidemiological and pathological observations.

The repeated coexistence of hemorrhagic and skeletal symptoms led to the conclusion that they were two different expressions of the same disturbance. It therefore seemed probable that the disturbance of the growth zones of the bones is due to the violent hemorrhagic phenomena seen there. It is significant that this malacic achondroplasia develops with a normal proportion of manganese in the bone ash, and a normal phospho-calcium ratio in the blood. The proportions of minerals and vitamins in the feed excludes the possibility of rickets or perosis. The juxta-articular lesions are similar to those described in turkey poults, sometimes in the chicken, under the name of "enlarged-hock-disorder," particularly when there is associated osteoporosis.

Thus the symptoms are similar to those described by Hunt and Blaylock (1953), Sherwood and Sloan (1953), and Butters and Scott (1956). But the analogy ends here because the etiology of "enlarged-hock-disorder" is attributed to the diet and an as yet undetermined mineral substance, and there is no mention of hemorrhages in soft or bony tissues.

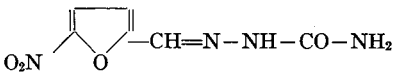
One observation seemed of primary importance: *the feeds, in these flocks, all contained nitrofural supplements.*

EXPERIMENTAL RESULTS

A series of experiments were performed, therefore, based on this observation. After 3 weeks on nitrofural-free mashes, permitting the sick birds to recover, nitrofural was again added to the feed *in doses of under 1 centigram per kilogram.* This immediately caused a change in the general condition. Within 24 hours, an intense anemia was observed, and locomotor difficulties were such that the birds refused to move. By the fourth day, most

of the birds were dead and autopsies showed the acute lesions of the intoxication. When the nitrofural was withdrawn from the feed 8 to 10 days after the beginning of the experiment, there was a rapid regression of the symptoms. Readdition of nitrofural did not cause the hemorrhagic and motor syndrome unless the toxic factor had been removed from the ration for a period of 10 to 20 days. The birds that did not die during the 8 to 10 days of nitrofural feeding seemed able to tolerate ever-increasing quantities (up to 1 gram per day) without showing any symptoms other than the anemia and the skeletal deformations. The experiment can be repeated, taking into consideration the refractory period following the administration of nitrofural for a few days. When an exceptional bird manages to survive succeeding experiments (and we have only seen one) and reaches the age of 4 or 6 months, its ability to react to the ingestion of the substances seems to diminish, and only the anemia can be shown clinically.

Looking at the furmula of nitrofural, which is as follows:



it was concluded that the part responsible for the hemorrhagic effects was the *semi-carbazone* chain. Therefore the experiments were repeated, using, instead of nitrofural, another drug, named Tébacyl (benzal-thiosemicarbazone urein), the formula of which contains the semicarbazone group. Given in doses of 10 to 23 mg. per day, it induced exactly the same syndrome as that previously observed with nitrofural.

This shows that *the semicarbazone radical is responsible for the toxic effects of nitrofural.*

In human medicine there is an allergic-type purpura due to an hemopathy, provoked by the repeated administration of thio-semicarbazones (Boivin, 1957). The analogy is striking: it can be confirmed by studying the variations in the hemograms of chickens under the influence of nitrofural.

When the birds are in good health and appear to have recovered after spontaneous or experimental hemorrhagic intoxication, a blood count shows over 3 million red corpuscles per cubic mm. of blood. The blood platelets fluctuate around 160,000, but this count may go as high as 350,000 for a few days after toxicity experiments. The differential blood count is 60 lymphocytes, 1 to 3 monocytes, 0 to 2 eosinophils, and about 40 pseudoesiniphils.

When hemorrhages occur, a sudden spectacular lowering of the blood platelets is seen. They fall to 90,000, then 60,000, 50,000, 35,000, and even less than 20,000 per cubic mm. The thrombocytopenia is manifest, and it is accompanied by a marked anemia (less than 1 million red blood corpuscles), and a modification of the white cell count towards a relative hyperlymphocytosis due to a decrease in the granulocytes, giving the following averages:

Pseudoeosinophils	less than 10
Lymphocytes	more than 85
Eosinophils	0 to 2
Monocytes	0 to 3

Examination of the bone marrow is significant in evaluating the importance of the hemopoietic disorders. Macroscopically it is decolored and fatty despite the blood effusions, which, in certain sites, may modify its aplastic aspect.

Histologically, there is rarefaction of myeloid tissue, and very few stem cells are seen. There are some apparently normal myelocyte- and erythrocyte-islands

and the impression is that granulocyte production has ceased, by an effect of toxic "sideration," rather similar to that found in human allergic purpuras.

CONCLUSION

It appears therefore that the hemorrhagic syndrome observed should be classified as a *thrombocytopenia with accompanying agranulocytosis*. The determination of the reactional mechanism in experimental subjects shows that the symptoms are of an *allergic nature*. In order to produce the pathological reaction a preliminary sensitization with a semicarbazone group substance is necessary, and after a certain refractory period, administration of a substance of the same group, even given in a very small dose, is immediately followed by the hemorrhagic condition.

Therefore, it would seem that these abnormalities can be avoided if semicarbazone-group medicines are given for a limited period only, and *continuously during that period.*

At present an attempt to establish the period of sensitization is being made, as well as a study of the possible influence other feed components may have, in particular, the proteins.

SUMMARY

Nitrofural is capable of producing a thrombocytopenic, hemorrhagic syndrome, by provoking a rapid, brutal, hemopoietic disturbance. The toxic factor acts after a preliminary sensitization period, during which time there are no clinical symptoms. This is followed by a clinical phase, and then a refractory period. In all probability an allergic mechanism is responsible.

The toxic factor of the drug is the semicarbazone radical, and its action is specific.

The hemorrhages are generally found in the conjunctive tissue, the serosae, and the bone growth-zones, resulting in either rapid death or poor growth accompanied by acute malacic and chondrodystrophic conditions.

Nitrofural should not be administered to growing birds until consideration has been given to its possible pathogenic action.

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