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CHARACTERIZATION OF GOSSYPOL INDUCED ANEMIAS

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ABSTRACT.—A study was made using male rats to determine the possible existence of a relationship between ingested gossypol concentration and the anemia types produced. The dietary levels were: free gossypol, 0.088 and 0.176 per cent; and bound gossypol, 0.055 and 0.110 per cent. The periods of feeding were 7, 14, 21, and 28 days. High levels of free gossypol resulted in hypochromic anemia in animals fed for 28 days. The results showed that the levels of hemoglobin, blood iron and liver iron were inversely related to the levels of free gossypol in the diet and to the length of time the diets were fed.

It has been reported that cottonseed meal is the second most important protein concentrate in the United States in volume of production, and the second most important source of supplementary protein for animal feeding. Nevertheless, limitations have been imposed on the amount of cottonseed meal which can be incorporated into animal feeds. These limitations are due to the presence in cottonseed of a phenolic pigment, gossypol, which is highly toxic to non-ruminant animals.

This toxic substance has been shown to accumulate in the organs of animals and its toxic effects are expressed in a wide array of pathological conditions (e.g., hemorrhagic intestines, hydrothorax, edema of pulmonary tissues, congestion of splanchnic organs, drastic weight loss and anemia).

Danke and Tillman (1965) reported that the ingestion of gossypol by non-ruminant animals will induce anemia, and that a high quality protein diet supplemented with iron can alleviate the anemic response. Their results suggest that gossypol may produce different anemias in the time interval following sustained gossypol ingestion. One type of anemia resulting from an immediate and direct effect of gossypol on the circulating red blood corpuscles may be hemolytic anemia. The other type of anemia (microcytic-hypochromic) occuring later in time may be due to the gossypol blockage of the absorption of some erythropoietic factor such as iron from the gastrointestinal tract or the impairment of erythropoiesis through the cumulative action of gossypol on erythropoietic processes elsewhere in the body.

This study was undertaken to clarify the possible existence of a relationship between the ingested gossypol concentration and the anemia types produced.

MATERIALS AND METHODS

Male rats (28 days of age) of the Holtzman strain were housed individually under uniform conditions of light (10 hr light, 14 hr dark) and temperature (68-72 F). Animals were randomly divided into three diet groups. The control diet (Diet 1) consisted of ground commercial rat chow. Diet 2 consisted of 90 per cent rat chow and 10 per cent ground cottonseed meats (0.088% free gossypol, 0.055% bound gossypol). Diet 3 consisted of 80 per cent rat chow and 20 per cent ground cottonseed meats (0.176% free gossypol, 0.110% bound gossypol).

Each of the three diet groups was represented by 64 animals. The animals in each major diet group were also randomly subdivided into four groups of 16 animals, each subgroup corresponding to feeding intervals of

TABLE 1.—Blood and liver data on rats fed cottonseed meal rations[a]

Days on Diet	7			14			21			28		
Diets	1	2	3	1	2	3	1	2	3	1	2	3
RBCx10^6/mm^3	6.71 ±0.17	5.95 ±0.13[b]*	6.74 ±0.24	6.99 ±0.16	7.01 ±0.12	7.28 ±0.24	7.59 ±0.23	7.16 ±0.22	7.21 ±0.45	7.39 ±0.14	7.70 ±0.14	6.90 ±0.34
Packed cells, %	42.88 ±0.56	41.06 ±0.57*	41.10 ±0.41*	44.20 ±0.44	45.23 ±0.43	44.87 ±0.57	48.21 ±0.72	46.68 ±0.40	45.38 ±0.58	47.54 ±0.56	48.03 ±0.45	46.25 ±1.46
Hb,g/100cm^3	13.05 ±0.75	13.36 ±0.22	13.29 ±0.28	14.02 ±0.16	14.25 ±0.17	14.87 ±0.15	15.13 ±0.30	14.49 ±0.24	14.38 ±0.25	17.20 ±0.52	16.32 ±0.68	11.59 ±0.96*
Mean corpuscular vol , μ^3	64.57 ±2.09	69.44 ±1.76	62.23 ±2.53	63.48 ±1.15	64.69 ±0.93	62.26 ±2.31	64.09 ±1.41	65.90 ±1.60	65.94 ±4.17	64.55 ±1.08	62.78 ±1.18	68.14 ±2.52
Mean corpuscular Hb, $\mu\mu$g	20.70 ±0.77	22.55 ±0.45	19.95 ±0.61	20.13 ±0.46	20.39 ±0.30	20.58 ±0.64	20.09 ±0.46	20.45 ±0.55	20.81 ±1.29	23.35 ±0.74	22.16 ±0.57	16.95 ±1.28*
Mean corpuscular Hb conc , %	32.00 ±0.30	32.66 ±0.78	32.40 ±0.88	31.74 ±0.36	31.52 ±0.27	33.18* ±0.32	31.40 ±0.52	31.06 ±0.48	31.72 ±0.53	36.17 ±0.97	35.37 ±0.57	25.29* ±2.06
Blood Fe, mg/100 ml	35.10 ±0.96	31.26* ±0.98	31.17* ±1.32	34.05 ±0.91	35.72 ±0.69	33.66 ±1.23	37.69 ±1.72	35.05 ±1.06	31.55* ±0.96	39.94 ±1.03	40.74 ±1.31	32.57* ±1.89
Liver Fe, ppm/g wet liver	140.99 ±5.00	129.69 ±4.81	124.04* ±5.20	141.07 ±3.88	144.45 ±5.28	133.28 ±5.10	156.05 ±6.87	141.40 ±4.77	122.48* ±4.25	157.36 ±6.69	165.03 ±6.81	124.85* ±6.55
% NaCl at which 50% hemolysis occurs	0.3595 ±0.0029	0.3863* ±0.0006	0.3566 ±0.0033	0.3735 ±0.0018	0.3644* ±0.0056	0.3753 ±0.0018	0.3796 ±0.0031	0.3930* ±0.0013	0.3950* ±0.0016	0.3812 ±0.0032	0.3903* ±0.0023	0.4034* ±0.0031

[a] Mean values ± SE

[b] * $P < 0.05$

7, 14, 21, and 28 days, respectively. Upon termination of each feeding interval blood was collected from representative animals and processed for total red blood cell count (RBC), hemoglobin content (Hb), percentage of packed cells, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell osmotic fragility (Swallen, 1964), and blood iron concentration (Natelson, 1961). Livers were excised and stored at -20 C until analyzed for total iron content (Sandell, 1959).

Results

Results of blood values and liver iron are recorded in Table 1. The erythrocyte count of control animals increased gradually to a maximum count by the end of the third feeding week. A slight decrease at the end of the fourth feeding week is indicated. Diet 2 animals exhibited a decrease in erythrocyte count at the end of the first feeding week followed by a gradual increase through the remaining feeding periods. An increase in erythrocyte count through two weeks of feeding is indicated by Diet 3 animals, with a gradual decline during the third and fourth feeding weeks.

Hematocrit values among control animals showed a similar trend and support the observed increase in erythrocyte numbers during the first three weeks of the feeding period and the observed leveling off thereafter. Hematocrit values for animals on experimental diets generally showed an increase after the first week on diets. The hematocrit values of animals fed experimental diets are significantly lower than the control value at the end of one week on diets. Among Diet 2 animals this decreased hematocrit value may be due to the significantly lower erythrocyte count.

The average hemoglobin concentration of the blood among control animals was found to increase progressively throughout the four week feeding period, and can be accounted for, at least during the first three weeks, to the increase in erythrocyte numbers. In the fourth week of feeding, however, the erythrocyte population did not increase nor did the mean corpuscular volume. Therefore, the increase in hemoglobin during the fourth week must be accounted for by an increased corpuscular hemoglobin concentration. The mean corpuscular hemoglobin concentration is shown to increase between the third and fourth weeks and supports the foregoing relationship. Among Diet 2 animals the average hemoglobin concentration of the blood exhibited a progressive increase throughout the four week feeding period. The average hemoglobin concentration of Diet 2 animals at one week on diet remained within normal ranges although the erythrocyte population was significantly decreased. This is accounted to the increase in mean corpuscular volume and mean corpuscular hemoglobin at this feeding period. Hemoglobin content of animals on Diet 3 showed a similar trend through the first 14 days on diet and then decreased rapidly at 21 and 28 days on diet. A decrease in mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration is also shown among Diet 3 animals at the end of the fourth feeding period.

Mean corpuscular volume among control animals remained stable through the four feeding periods while it gradually decreased among Diet 2 animals. Among Diet 3 animals through the first two feeding periods, the mean corpuscular volume remained stable and gradually increased to a maximum at the end of the fourth feeding period.

Generally after the first feeding period, erythrocytes in all diet groups displayed a decreased resistance to hypotonic solutions. At the first, third and fourth feeding periods Diet 2 animals were significantly less resistant to hypotonic lysis. Diet 3 animals exhibited significantly less resistance at the third and fourth feeding periods.

Mean blood and liver iron were significantly decreased at the first, third and fourth feeding periods among Diet 3 animals while these values were significantly decreased among Diet 2 animals at the end of the first feeding period.

Discussion

Menaul (1923) determined that gossypol exerts a hemolytic effect on erythrocytes. The presence of a lower erythrocyte count among animals maintained on Diet 2 for seven days suggests the presence of some hemolytic action by gossypol. However, the absence of significantly lower erythrocyte counts at longer feeding periods on Diet 2, and the absence of this condition among animals fed the higher levels of free gossypol in Diet 3 indicate that this effect was not persistent throughout the investigation. Although the animals were in their rapidly growing phase throughout most of this study, it appears that the rations contained adequate protein to compensate for the deleterious effect of gossypol on erythrocyte hemolysis. The increased corpuscular volume at seven days on diet for animals that ingested Diet 2 indicates a higher proportion of younger erythrocytes. This effect would support a compensatory response to a hemolytic agent.

Erythrocytes from animals fed Diet 3 for 28 days were hypochromic which is indicative of an iron deficiency that could result from inadequate absorption of iron, a deficiency in dietary iron or an excessive demand for iron. The blood and liver iron levels among these animals at this feeding were significantly lower. This would support the finding that the iron stores were inadequate to maintain adequate hemoglobin synthesis. It has been known for years that soluble iron salts inactivate free gossypol in the diet (Gallup, 1928). It has been suggested that the iron forms an insoluble gossypol-iron complex in the intestinal tract that is not absorbed. Shieh *et al.* (1968) have suggested in their chelation studies the formation of a 1 to 2 molar ratio of gossypol to ferrous ions. The lower blood and liver iron values exhibited among Diet 3 animals after 28 days on diet indicate the possibility that the free gossypol in the diet chelated with iron in the intestinal tract and caused a reduction in iron absorption.

The increased fragility of erythrocytes among animals fed experimental diets for 21 and 28 days provides evidence of spherocytosis and/or abnormalities in the shape of the erythrocytes. When an erythrocyte is placed in a hypotonic solution it will become increasingly spherical in shape and eventually lyse. Spherocytes (cells that are initially spherical) would expand very little before the cell membrane is stretched and lysis occurs. Thus, spherocytes would have less resistance than normal cells to hypotonic solutions. It has been determined that spherocytes possess a basic intracellular defect in phosphorylation and consequently are less able to maintain their integrity than normal cells (Motulsky *et al.*, 1955). Since gossypol is lipophilic and may penetrate the erythrocyte membrane by diffusion, it may induce an abnormal cell shape through the disruption of some aspect of erythrocyte metabolism (Abou-Donia and Lyman, 1969).

It is also possible that the decreased resistance to lysis of erythrocytes among experimental animals may be due to a direct effect of gossypol on the erythrocyte stroma. The lipophilic gossypol may change the integrity of the cell membrane and decrease the resistance of the erythrocytes to hypotonic solutions.

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