Figure 3. Chromatograms of some root crops analyzed by the developed method. Chromatograms were obtained on different days and are not comparable. Attenuation, 2×. A, 4 ng propazine; B, 6 ng simazine; C, 4 ng atrazine; D, 6 ng sencor.

able for the quantitative glc–CCD analysis for triazines in root crops. With the methanol extraction methods, the hexane partition and column cleanup were not required and thus a considerable saving of analysis time resulted. Methanol was chosen as the extraction solvent because it filtered rapidly from the macerate and produced no emulsions in the partitions. Chloroform or dichloromethane was chosen over the other partitioning solvents because of fewer impurities extracted. These extracts were dried over anhydrous Na_2SO_4, evaporated to dryness, and dissolved in ethyl acetate for glc analysis. Although the extracts were yellow, no significant interferences were encountered. Figure 3 illustrates results from several vegetables at 0.2–0.02 ppm using the above method. Table I shows the recoveries obtained using this method for a number of triazines down to 0.02 ppm. The triazines are essentially quantitatively extracted from the crops down to the 0.02-ppm level. At the lower concentrations, it was possible to take a 20-ml aliquot of the methanol for partitioning and analysis. However, larger volumes required evaporation to about 10–20 ml before partitioning. The methanol extracts and the final ethyl acetate extracts were kept for 2 weeks

at $-2°$ with no effect on quantitative results for any of the herbicides studied.

CONCLUSIONS

The Coulson electrolytic conductivity detector has proven to be very useful for the analysis of triazines in root crops. The detector makes hexane partitioning and column cleanup unnecessary. Analysis time is decreased greatly while quantitation for low levels of triazine (<0.1 ppm) is equal to other detection methods.

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Hematological Effects of Injected Gossypol and Iron in Rats

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Studies designed to determine the hematological effects of intraperitoneally injected gossypol acetic acid and iron in rats were carried out. A total of 185 animals were divided into four groups. One experimental group was injected with corn oil containing 2 mg of gossypol acetic acid/ml at a dosage level of 1.5 mg/100 g of body weight; control animals were injected with 1.2 ml of corn oil. Another group was injected with the same dosage of gossypol and intramuscularly with 2 ml of iron sorbitex containing 50 mg of iron/ml; control animals were injected with 1.2 ml of corn oil and 2

ml of iron sorbitex. Gossypol injection resulted in an increase in erythrocytes, packed cell volumes, and hemoglobin concentrations during the first 7 days of postinjection; these same hematologic parameters were reduced below normal values by the 14th day postinjection. On the other hand, iron-injected animals had a normal erythrocyte population at the 14th day postinjection. Free and bound gossypol accumulated in the livers of gossypol-injected animals and was progressively eliminated as the postinjection time was increased.

A toxic effect of ingested gossypol on some blood constituents of nonruminant animals has been established. Gossypol has a hemolytic effect on erythrocytes and inhibits the dissociation of oxyhemoglobin (Menaul, 1922,

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1923). Pigs which were fed high levels of gossypol developed a hypoprothrombinemia and a decreased hemoglobin concentration (Clawson et al., 1962; Harms and Holley, 1951). Rats which were fed high levels of free gossypol for 28 days developed a microcytic-hypochromic anemia (Danke and Tillman, 1965). Supplemental iron in the diet alleviated this condition.

The lethal level of injected gossypol for rats (Gallup, 1931) and factors that alter the toxicity of injected gossypol have been established (Clawson et al., 1962). It has been confirmed that biliary secretion constitutes the major route of elimination of intravenously injected gossypol in swine (Albrecht et al., 1972).

However, there are no data relative to the toxic effect(s) of injected gossypol on blood parameters. This study was designed to determine the possible toxic effects of injected gossypol on a number of blood parameters and to determine the effect of injected iron on the blood parameters of gossypol-injected rats.

MATERIALS AND METHODS

A total of 185 male rats of the Holtzman strain (36 days old, 136–141 g) were housed individually in metabolism cages under uniform conditions of light (10 hr of light, 14 hour of dark) and temperature (69–72°F). Food and water were provided ad libitum. The diet consisted of ground commercial rat chow. Food consumption for each rat was determined daily and its body weight recorded twice weekly.

The animals were randomly divided into four injection groups: C-1, corn oil, 70 animals; E-1, corn oil plus gossypol, 75 animals; C-2, corn oil plus iron sorbitex, 20 animals; E-2, corn oil plus iron sorbitex plus gossypol, 20 animals.

The initial 2 weeks of the study constituted a preinjection-acclimation period. Following the preinjection-acclimation period, animals were injected intraperitoneally with 1.2 ml of corn oil. E-1 animals were injected intraperitoneally with corn oil containing 2 mg of gossypol acetic acid/ml at a dosage level of 1.5 mg/100 g of body weight. E-2 animals were injected intraperitoneally at the same gossypol dosage level as the E-1 animals and intramuscularly with 2 ml of iron sorbitex containing 50 mg of iron/ml. C-2 animals were injected intraperitoneally with 1.2 ml of corn oil and intramuscularly with 2 ml of iron sorbitex.

Animals within the four injection groups were randomly assigned to sampling subgroups corresponding to the number of postinjection days lapsed prior to blood sampling. Tail blood samples were obtained from the rats of each sampling group in C-1 and E-1 animals on days 1 through 7, and on days 14 and 21, postinjection. Blood sampling in C-2 and E-2 animals was restricted to days 14 and 21, postinjection. Each sampling group was comprised of not fewer than five rats.

The following hematological parameters were investigated: total red blood cell count (RBC), hemoglobin concentration (Hb), percentage of packed cells (Hct), percentage of reticulocytes, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white cell count (WBC), and white cell differential count.

Livers were excised for the histochemical determination of iron content (Lillie, 1965) and for the analysis of free and bound gossypol content (Smith, 1965).

Statistical analyses of the data were conducted using the Student's t test at a significance level of $p < 0.05$.

RESULTS

There were no significant differences in daily weight gain, daily food intake, and food consumed per gram gain among all groups during the preinjection-acclimation period of 2 weeks. Animals in both experimental groups lost weight during the first week postinjection. At the end of the second week postinjection, the animals in the experimental groups exhibited a positive weight gain. However, the weight loss occurring in the first week of postinjection was never recovered. The daily weight gain and daily food intake were significantly less among the experimental ani-

mals after 14 days postinjection. E-1 and C-1 animals exhibited a similar weight gain at 21 days postinjection.

Erythrocyte data are presented in Table I. An increase in red blood cell counts among E-1 animals was demonstrated in the interval of days 1 through 7, postinjection. The increases were significant at days 1 and 3, postinjection. E-1 animals exhibited a significant decrease in the red blood cell count at 14 days postinjection. The mean red blood cell count for E-1 animals was within a normal range at 21 days postinjection.

The hematocrit and hemoglobin values for this same group of experimental animals followed a similar trend and generally support the observed changes in red blood cell counts.

E-2 animals exhibited an increase in red blood cell counts, hematocrit values, and hemoglobin concentrations at 7 days postinjection. The red blood cell counts were within a normal range at 14 days postinjection. Hematocrit values and hemoglobin values, however, were significantly less than control values.

Mean corpuscular volume and mean corpuscular hemoglobin values were within normal ranges among all experimental groups. With the exception of the significantly reduced mean corpuscular hemoglobin concentration of E-1 animals at 14 days postinjection, values for this parameter were within normal ranges for E-1 and E-2 animals.

The percentages of reticulocytes in the peripheral circulation of E-2 animals were generally lower than the control values during the first 7 days postinjection.

Total leucocyte counts were significantly increased in both groups of experimental animals at 14 days postinjection. The lymphocyte–neutrophil ratio was significantly reversed among E-1 animals on days 1 through 7, and at day 14, postinjection. At 7 and 14 days of postinjection, this ratio was significantly reversed among E-2 animals. Among all experimental animals the percentages of eosinophils and monocytes were not significantly different.

The results from liver analyses for gossypol (Table II) indicate a general decrease in the concentration of free and bound gossypol among E-1 animals as the postinjection period increased. At 7 days postinjection the free and bound gossypol accumulation in the livers of E-1 animals was lower ($p < 0.05$) than that of E-2 animals. At 14 days postinjection the bound gossypol concentration among E-2 animals was lower ($p < 0.05$) than at 7 days postinjection.

Histochemical analyses for iron revealed a gradual decrease in liver iron among E-1 animals from 1 through 7 days of postinjection. The lowest level was observed at 14 days postinjection among E-1 animals. An increase in iron concentration was revealed among E-1 animals at 21 days of postinjection. The liver iron levels were higher among E-2 animals at 7 and 14 days postinjection than for E-1 animals.

DISCUSSION

The experimental animals autopsied on postinjection days 1 through 7 were found to have a marked accumulation of fluid in the peritoneal cavity. The excessive peritoneal fluid was attributed to the loss of fluid from the vascular compartment into the peritoneal cavity. Smith (1957) described a similar occurrence of excessive fluid in the peritoneal cavities of pigs which had ingested free gossypol. The loss of vascular fluid probably accounts for the increase in the red blood cell counts of E-1 and E-2 animals during the postinjection period of days 1 through 7. Hypovolemia could be accompanied by abnormally high erythroid values even in the presence of a normal red blood cell count. As a result of the reduction in the plasma volume by loss of fluid into the peritoneal cavity, the red cell mass would be concentrated and the peripheral erythroid values (hemoglobin concentration, hematocrit value, and red blood cell count) would be increased until a normal plasma volume was established.

Table I. The Effects on Blood Components of Rats Following Injections of Corn Oil, Corn Oil plus Iron, and Corn Oil plus Iron plus Gossypol[a]

	Postinjection time, days								
	1	2	3	4	5	6	7	14	21
RBC × 10⁶/mm³	8.04 ± 0.41	8.12 ± 0.19	7.61 ± 0.26	8.37 ± 0.28	8.03 ± 0.20	8.31 ± 0.15	7.70 ± 0.24	8.21 ± 0.25	8.58 ± 0.1
	9.19 ± 0.30[b]	8.64 ± 0.18	8.72 ± 0.27[b]	9.12 ± 0.25	8.61 ± 0.21	8.92 ± 0.41	8.57 ± 0.38	6.90 ± 0.41[b]	8.21 ± 0.36
							8.45 ± 0.30	8.54 ± 0.20	
							9.40 ± 0.26[b]	8.05 ± 0.52	
Hct, %	48.6 ± 2.5	45.6 ± 0.8	44.3 ± 0.8	47.0 ± 0.8	45.8 ± 1.0	45.1 ± 1.0	45.4 ± 0.4	47.3 ± 1.1	48.3 ± 0.7
	55.2 ± 1.5[b]	48.2 ± 0.8	51.9 ± 1.9[b]	49.1 ± 0.7	49.2 ± 0.8[b]	49.8 ± 2.6	48.2 ± 1.5	41.3 ± 2.1[b]	48.8 ± 1.9
							45.5 ± 0.7	46.9 ± 0.6	
							50.0 ± 1.0[b]	43.2 ± 1.3[b]	
Hb, g/100 cm³	16.20 ± 1.10	15.68 ± 0.22	14.61 ± 0.33	16.20 ± 0.17	15.13 ± 0.54	15.50 ± 0.57	14.90 ± 0.20	15.93 ± 0.40	16.46 ± 0.29
	18.28 ± 0.80	17.00 ± 0.41[b]	17.45 ± 0.58[b]	17.10 ± 0.30[b]	16.55 ± 0.32[b]	16.97 ± 0.96	16.30 ± 0.69	13.01 ± 0.74[b]	16.19 ± 0.64
							15.47 ± 0.17	16.00 ± 0.20	
							17.00 ± 0.24[b]	14.64 ± 0.52[b]	
Reticulocytes, %	3.2 ± 0.6	2.9 ± 0.9	2.5 ± 0.6	3.2 ± 0.6	2.1 ± 0.3	2.7 ± 0.7	2.8 ± 1.0	2.9 ± 0.7	1.3 ± 0.2
	1.8 ± 0.4	2.1 ± 0.4	1.6 ± 0.3	1.8 ± 0.4	0.8 ± 0.1[b]	1.5 ± 0.5	0.8 ± 0.4[b]	4.3 ± 1.4	2.1 ± 0.4
							3.6 ± 0.6	3.4 ± 0.4	
							1.5 ± 0.4[b]	2.9 ± 0.7	
WBC × 10³/mm³	15.98 ± 0.83	20.44 ± 1.84	21.20 ± 3.65	17.72 ± 3.97	17.94 ± 1.71	18.09 ± 3.08	16.82 ± 1.80	16.25 ± 1.28	21.84 ± 1.59
	13.31 ± 1.32	20.79 ± 1.43	18.53 ± 2.97	19.12 ± 1.79	20.53 ± 2.14	18.90 ± 4.63	14.18 ± 3.07	24.25 ± 3.10[b]	17.06 ± 4.45
							16.39 ± 1.10	19.82 ± 1.02	
							12.46 ± 1.09[b]	22.66 ± 1.72[b]	
Lymphocytes, %	93.0 ± 2.8	85.8 ± 2.3	86.2 ± 3.9	88.2 ± 4.0	88.2 ± 2.1	91.0 ± 2.3	86.1 ± 2.2	83.4 ± 3.1	87.4 ± 2.0
	48.4 ± 4.8[b]	51.6 ± 5.1[b]	47.9 ± 4.9[b]	49.7 ± 5.7[b]	51.1 ± 6.5[b]	63.4 ± 7.9[b]	43.8 ± 11.9[b]	62.4 ± 6.0[b]	87.8 ± 3.0
							86.6 ± 1.7	88.6 ± 1.9	
							55.8 ± 10.9[b]	54.3 ± 5.0[b]	
Neutrophils, %	6.4 ± 2.2	13.4 ± 3.3	12.8 ± 3.8	11.4 ± 4.1	11.6 ± 1.6	7.8 ± 1.9	11.9 ± 2.0	15.8 ± 3.1	10.7 ± 2.0
	51.3 ± 4.9[b]	48.0 ± 5.2[b]	51.9 ± 5.1[b]	49.8 ± 5.7[b]	48.3 ± 6.7[b]	36.3 ± 8.0[b]	55.4 ± 12.7[b]	34.8 ± 6.4[b]	11.0 ± 3.0
							11.8 ± 1.5	10.8 ± 2.9	
							44.0 ± 7.2[b]	45.3 ± 5.0[b]	

[a] From top to bottom within each parameter for a postinjection period the mean values (±SE) represent data from the following groups: corn oil, corn oil plus iron, corn oil plus gossypol, corn oil plus iron plus gossypol. [b] p < 0.05.

Table II. Gossypol Content of Livers from Rats Injected with Corn Oil plus Gossypol or Corn Oil plus Iron plus Gossypol[a]

	Postinjection time, days								
	1	2	3	4	5	6	7	14	21
Free gossypol	15.8 ± 2.9	12.2 ± 2.2	4.9 ± 1.6	3.5 ± 1.5	0.0	0.0	0.8 ± 0.7	1.1 ± 0.9	2.7 ± 1.8
							6.3 ± 2.4	2.5 ± 1.7	
Bound gossypol	81.3 ± 14.5	43.7 ± 5.5	44.7 ± 7.2	62.5 ± 9.0	48.0 ± 11.2	6.9 ± 0.1	28.8 ± 7.8	4.5 ± 3.2	3.4 ± 1.6
							41.9 ± 5.2	6.9 ± 4.3	

[a] From top to bottom within each form of gossypol the mean values ±SE (μg of gossypol/g of wet weight) represent data from the following groups: corn oil plus gossypol and corn oil plus iron plus gossypol.

The percentage of circulating reticulocytes is generally considered to reflect the hemopoietic activity of the bone marrow and is usually observed to increase following hemorrhage or red blood cell hemolysis. Since the percentage of reticulocytes in the peripheral blood was not elevated during the first 7 postinjection days, it would appear that the increase in the red blood cell count and peripheral erythroid values of the experimental rats during this time was not due to an increase in the red blood cell population. In addition, it is indicated that the injected gossypol did not produce a significant red blood cell hemolysis in the first 7 postinjection days.

A subsequent and significant reduction in the red blood cell count, hematocrit, and hemoglobin concentration of E-1 animals by 14 days postinjection may be the result of a combination of the following: an increase in the plasma volume, a gossypol-induced red blood cell hemolysis (Menaul, 1922, 1923), and a gossypol-induced inhibition of hematopoiesis (Clawson et al., 1962). However, the lack of a significant increase in the percentage of reticulocytes and hemoglobin in the urine among the experimental animals at 14 days postinjection suggests that the reduction in the red blood cell count, hematocrit, and hemoglobin concentration was not due to the hemolysis of red blood cells. Because of the significant reduction in the red blood cell count, hematocrit, and hemoglobin concentration, the peripheral erythroid values would be expected to decrease, and continue to decrease, until the causes had been alleviated. The establishment of normal erythroid values by E-1 animals at 21 days postinjection, from the increased values of postinjection days 1 through 7 and subsequent reduced values by postinjection day 14, would indicate that an alleviation of causative factors occurred.

It has been known for several years that iron salts can alleviate gossypol toxicity, apparently by the formation of a chelation complex between ferrous ions and gossypol (Jonassen and Demint, 1955; Muzaffaruddin and Saxena, 1966; Shieh et al., 1968). Iron sorbitex, injected with gossypol into E-2 animals, proved to be of some benefit in maintaining a normal red blood cell mass through postinjection day 14. Although the hemoglobin concentration and hematocrit values were below their normal ranges,

these same values were greater than comparable values for E-1 animals. These results would indicate that injected iron had the capability of inactivating free gossypol. The probable formation of an inactive iron-gossypol complex in E-2 animals reduced the toxic effect of gossypol that was exhibited among E-1 animals at 14 days postinjection.

The neutrophilia observed in both E-1 and E-2 animals during postinjection days 1 through 7 and at day 14 may possibly be the result of an inflammatory response by the animals following the introduction of foreign chemical agents into the peritoneal cavity.

Following the injection of gossypol, it is apparent that free and bound gossypol decreased progressively as the postinjection time increased. This indicates that this highly vascular organ has an important role in the accumulation and elimination of injected gossypol. The higher liver iron levels exhibited by E-2 animals at 7 and 14 days postinjection may account for their higher bound gossypol levels observed for these same postinjection periods. It is possible that more gossypol would be chelated with iron if the iron levels are higher.

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