components separated electrophoretically by the preparative method still showed a marked absorption band at 2600 Å, but none at 3600 Å. Ninety percent methanol leached, lyophilized ragweed pollen extracts (MLL) show a marked depression of the 2600 Å and the 3600 Å bands. Biological activity of the essentially pigment-free preparation is identical with the preparation containing all of the pigment thus confirming our previous point of view that the pigments do not contribute substantially to the biological activity. Electrophoretic analysis reveals that a new substantially colorless major component is formed, on removal of the pigment, with an electric mobility of approximately 0.3 μ per second at pH 7.4.

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Effect of 200 Roentgens Fractional Whole Body Irradiation in the Burro. (20848)

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In a previous investigation of the response of the burro to 400 r fractional whole body irradiation(1), it was observed that this species responded to such irradiation in the same manner as swine(2) and goats(3). In addition the burro demonstrated a pronounced hyperferremia one hour after the completion of the first 400 r exposure and this condition reached a peak on the sixth irradiation day. The present investigation was designed to compare the possible differences in response which might occur when the total daily dose was reduced and to compare the overall response with that seen after a single acute dose in the LD_{50} range.

Methods. Ten male burros were exposed to 200 r Co^{60} gamma ray whole body irradiation daily for 16 days using the multicurie irradiation site described by Wilding *et al.*(4). The radiation flux was approximately 48 r/hr. Control blood samples were obtained 3 times prior to irradiation and daily within one hour after the completion of each irradiation. Hemoglobin was determined colorimetrically by a modified Dupray method(5-7). Platelet counts were made with the method of Brecher and Cronkite(8) and the other hematological values were obtained by the methods of Wintrobe(9). Plasma iron values were determined by the method of Kitzes *et al.*(10) using 2 ml aliquots from plasma samples kept frozen until analyzed. Records were kept of daily body temperatures, food and water consumption and total body weight loss during the experimental period.

Results. General effects. During the first irradiation day the animals appeared normal, but were listless after 400 r and became more so with each subsequent irradiation. The

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first death occurred after 1200 r had been administered but nine of the animals received 2800 to 3200 r before dying. Irritation noticed during the initial sickness changed to a passive tolerant attitude and a pseudo-euphorism became apparent after the first irradiation week. The mean body temperature remained within normal limits (36.8-37.5°C) until 1000 r had been given, at which time a slight decrease was noted (35.7-36.8°C). A definite increase in temperature occurred and continued after 2000 r had been received (37.2-38.2°C) reaching a peak at 2600 r (37.8-39.5°C). Near death the temperatures in some animals were markedly below normal and none were recorded after the 2800 r dose. Anorexia and a reduced water intake were observed beginning upon the eighth irradiation day and continuing until exitus. The average body weight loss for the group was 2.98 kg/day. Buccal and gingival ulcerations, fecial swelling, salivation and twitching of the lips were common after the tenth irradiation day, at which time the breath of the burros was malodorous. Prolonged bleeding occurred at the veni-puncture site and blood flowed from the nares, mouth and small wounds during the 9th, 10th and 11th days. This bleeding, indicative of a blood coagulation defect, continued until exitus. Some of the animals had encephalitis-like symptoms consisting of non-directional walking and a spasmodic muscular twitching. All of them exhibited a locomotion defect characterized by a stilted walk, "popping" of the joints and "knuckling over" of other joints. Retraction of the testicles high into the scrotum and in some animals into the abdomen was noted at necropsy. There was no epilation but hyperesthesia, particularly noted by hand pressure on the back, was apparent prior to exitus. Diarrhea was not observed and equidae do not vomit. One burro (No. 301) did not confirm to the general syndrome but collapsed suddenly 43½ hours after the beginning of irradiation (400 r). Although helped to his feet, he never again was able to stand. He appeared to want to arise, eat and drink but could not. Consciousness remained until just before death, 140 hours after irradiation began (1200 r). Spasmodic muscular twitching of the

TABLE I. Effect of 200 r Fractional Gamma Ray Irradiation on Hematology and Plasma Iron of Burros. Mean values ± stand. dev.*

Constituent		Pre-irrad. control*	Irradiation days													
			1	2	3	4	5	6	7	8	9	10	11	12	13	14
Hemoglobin	$\bar{x}$	10.3	10.3	10.4	9.9	9.4	9.9	9.9	9.7	9.5	9.6	9.0	8.8	8.8	9.2	8.6
	±	.2	.2	.2	.3	.3	.3	.3	.1	.2	.3	.2	.2	.3	.2	.5
Hematocrit	$\bar{x}$	33.1	32.7	32.3	32.6	31.4	32.7	32.9	32.0	31.2	31.1	29.4	28.8	28.7	28.8	31.8
	±	.5	.9	.6	.1	1.0	.9	1.0	.5	.7	.9	.5	.3	.8	.7	1.0
Erythrocytes $\times 10^{-6}$	$\bar{x}$	6.31	6.33	6.19	6.22	5.6	6.2	6.3	6.1	5.4	5.9	5.6	5.5	5.5	5.4	6.0
	±	.88	.15	.82	.10	.2	.1	.2	.1	.3	.2	.1	.1	.2	.1	.2
Leukocytes $\times 10^{-3}$	$\bar{x}$	15.8	11.5	8.5	7.0	6.1	5.0	4.3	2.8	2.3	1.4	.8	.4	.3	.2	.1
	±	2.1	.5	.8	.4	.3	.3	.3	.3	.2	.3	.2	.1	.1	.05	.01
Platelets $\times 10^{-3}$	$\bar{x}$	515	567	537	497	446	431	414	415	386	371	295	272.0	152	78.	34
	±	15.0	26.0	22.0	15.0	16.	16.	14.	14.	11.	9.0	22.	23.	29.	17.	9.
Plasma iron, μg %		109.7	115.9	110.3	149.8	110.6	138.1	144.9	144.7	163.2	176.9	165.2	151.9	138.8	136.6	152.4
	±	4.4	5.1	4.8	12.6	7.1	5.2	6.6	4.6	7.6	6.1	7.7	6.1	5.4	4.4	12.2

* Pre-irradiation control values represent results from 27-30 samples. All others are from 9-10 animals except last day when there were 8.

head and neck was seen from the 43rd to the 100 hour. No gross changes were observed at necropsy.

Hematological effects. The effect of the irradiation on the hematological picture is given in Table I. In general there was no great effect upon hemoglobin concentration or the number of erythrocytes until a slight decrease appeared in both on the 8th irradiation day. The values seem to indicate that the total blood loss from hemorrhage was minor up to the 9th day. The hematocrit values changed only slightly throughout the experimental period. A leukopenia began after only one exposure and became more pronounced with each subsequent exposure. Inasmuch as differential counts were not performed, no information concerning the effect of irradiation on individual leukocytic components is available. The platelets showed a slow diminution in number, characteristic of the burro, until the 11th day when half the normal number were present. Thereafter the platelets disappeared at a rapid rate reaching 6% of normal on the 14th day. The decrease in the number of platelets coincided with the appearance of the hemorrhagic symptoms. The leukocyte counts for animal No. 301 have been omitted from Table I because this burro had abnormally high counts (18,260) prior to irradiation and leukocytosis on the third day (19,900). At exitus the value was 2750.

Effect on Plasma Iron. Table I gives the daily plasma iron values for all animals. There is a considerable spread in individual animal daily iron values but the mean values indicate a significant rise within one hour after the third dose (total 600 r) had been administered, with a mean peak value after 1800 r and a slight progressive decrease until exitus. However, this latter effect did not cause the plasma iron values to approach the mean control values. The majority of animals showed significantly elevated plasma iron values prior to death.

Discussion. The overall response of the burro to 200 r daily exposure to Co^{60} gamma ray whole body irradiation was similar to that observed with both 400 r fractional(1) and single LD_{50} acute whole body irradiation(11). However, certain differences between the responses to 200 r and 400 r were apparent. The former animals had a hyperthermia and bleeding syndrome, whereas the latter ones did not. The 200 r burros showed a progressive decrease in hemoglobin concentration, erythrocyte count and hematocrit value while the 400 r animals showed no effect on the first two values, and an increase in the last. This indicates a progressive anemia and hemodilution in the 200 r group and a hemoconcentration in the 400 r group. As would be expected, the leukocyte counts indicate a slower rate of destruction from 200 r than from 400 r. The response of the platelets to both amounts of radiation was the same at similar time intervals but the 400 r animals did not live long enough to show the pronounced decrease seen in both the 200 r and the acute LD_{50} series on the 12th day. The normal plasma iron values for the 200 r series were higher than in the 400 r series but the overall response was the same and both groups reached a peak after 1800 r had been administered. The significance of this increase in plasma iron is difficult to interpret because complete information is lacking regarding its source and there is no good correlation between its presence and lethality. However, increased plasma iron has been observed in irradiated rats(12), dogs(13), burros(1) and rabbits(14), and part of this increase in circulating iron has been shown to be ferritin liberated from the liver(15,16).

Summary. A study has been made of the response of the burro to 200 r fractional Co^{60} gamma ray whole body irradiation. The response is similar to but slower than that observed with a daily dose of 400 r. The response of the platelets to irradiation with 200 r daily corresponded most closely with that seen when an acute LD_{50} dose had been given and correlated with the bleeding tendency which developed on the ninth irradiation day. A significant hyperferremia was observed in the burro one hour after receiving a total dose of 600 r but the maximum response was not obtained until 1800 r had been administered. Most animals had significantly elevated plasma iron values prior to exitus.

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Inhibition of Brain Respiration *in vitro* by Bilirubin. Reversal of Inhibition By Various Means.*† (20849)

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Kernicterus is the yellow staining of areas of gray matter found at autopsy in infants dying of erythroblastosis fetalis(1). The prevention of kernicterus and its concomitant nerve cell damage by replacement transfusion therapy provides the chief justification for that procedure(2). Other conditions than hemolytic anemia affecting the newborn, and associated with jaundice, may also be complicated by kernicterus(3,4). Although the pigment as it is found within nerve cells has been identified as mesobilirubin, the toxic agent or agents remain unknown(5). The present research was undertaken to see if bilirubin itself could be a contributing factor to the death of nerve cells. Although clinical experience indicates that jaundice is well tolerated by adults, high serum levels of indirect bilirubin in neonatal life are associated with a high death rate stemming apparently from kernicterus(6), and follow-up studies of infants recovered from erythroblastosis show an association between severe jaundice and a later impairment of intelligence(7).

Material and methods. Rats weighing from 25 to 75 g were decapitated, their cerebral hemispheres removed and at once chilled and chopped with scissors into pieces less than approximately 2 mm in their largest diameter. From 200 to 300 mg of chopped brain were placed in each of 4 to 6 Warburg flasks, the air replaced with oxygen, and oxygen uptake at 24.6°C recorded for the first 40 to 50 minutes after temperature stabilization. The fluid bathing the brain consisted of an electrolyte solution either as such for controls or with bilirubin and other test substances dissolved in it. The composition of the fluid, before the addition of bilirubin and adjustment of pH was as follows: NaCl .126, KCl .0025, Na_2CO_3 .082, Buffer .008 molar (Tris-(hydroxymethyl)Aminomethane). After the test substances were dissolved, the control and

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