results in the percent uptake as given by the formula:

$$\frac{\text{Animal weight}}{100} \times 4.59 \times \text{Hematocrit} \times \frac{\text{CPM/cc of cell sample}}{\text{Total CPM injected}} \times 100 = \% \text{ dose in red cells}$$

Results. The graphs of the results obtained in series A are shown in Fig. 1, series B in Fig. 2, and series C in Fig 3. The control series results are superimposed on each graph. Each point in the graphs represents the average value of the 5 animals in the group except where there was unavoidable mortality due to cardiac puncture.

Discussion. The results obtained in these studies show that the radioactive iron red cell uptake curve is a sensitive indicator of x-irradiation damage to the bone marrow. They also confirm the histologic evidence of the sensitivity of erythroid tissue to x-irradiation.

It appears that the greatest depression in erythroid marrow activity occurs at a period of about one day after the irradiation since the groups labeled at this period show a much greater depression at the 5 and 25 roentgen levels.

Summary. 1. A method of demonstrating the depression of the erythroid portion of the bone marrow by x-irradiation with the use of Fe^{59} is presented.

2. Graphs of the red cell Fe^{59} uptake curve in rats at various dosage levels of total body x-irradiation and at varying times after irradiation are given.

We wish to extend our sincere appreciation to Doctor John H. Lawrence for his kindly interest and assistance in this work.

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Assay of Circulating Progesterone by Ultraviolet Spectroscopy. (17707)

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The extraction and analysis of progestin from the blood of mammals has been described by Bloch(1). Although the presence of circulating progestin was corroborated by subsequent investigators, the need for a sensitive and accurate method of assay was obvious. Bloch, utilizing the bio-assay technic of Corner and Allen(2), demonstrated the presence of less than 0.2 μg of progesterone per cc of pooled sow blood. Human pregnancy serum yielded less than 1.0 μg of progesterone per cc. Haskins(3), following the bioassay procedure described by McGinty *et al.*(4), estimated the circulating progestin level in human pregnancy serum at 0.13 μg per cc, expressed as crystalline progesterone. De Allende(5), found the circulating progestin level to vary between 0.06 μg and 2.5 μg per cc of serum in the *Macacus rhesus*, during the menstrual cycle. Hooker and Forbes(6-9), using the method devised by them detected whole blood progesterone concentrations between 4 and 8 μg per cc in rabbits, mice, a

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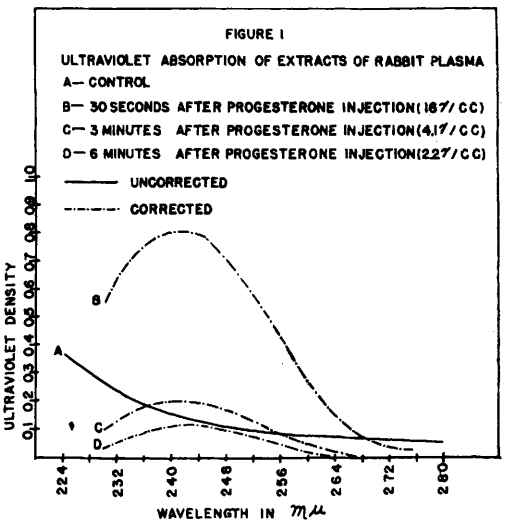
monkey and a pregnant woman. The higher progesterone content of blood reported by Hooker and Forbes indicated the possible applicability of ultraviolet analysis of ether extracts of serum for progesterone. The relatively high extinction coefficient of this steroid at 240 mμ renders it readily and accurately measurable in optimum concentrations of 1 to 20 μg per cc solvent (ethanol). Although contaminating substances alter the sensitivity, suitable blanks or controls overcome this difficulty.

A series of experiments were devised in an attempt to demonstrate circulating progesterone by ultraviolet analysis in the blood of pregnant women and in rabbits following the intravenous administration of this substance. Rabbits were also used as test animals in following the blood levels of testosterone and Δ-4 cholestenone after their intravenous administration. Each of the latter approximates the extinction coefficient of progesterone at 240 mμ.

Method. All progesterone determinations were made by ultraviolet analysis. The Beckman spectrophotometer was used with one cm square fused silica cuvettes. All substances analyzed were dissolved in 95% ethanol. Synthetic alpha progesterone with a melting point of 128°C was used. Blood was obtained by venipuncture from women patients whose reproductive status was known. The blood was oxalated and the plasma was expressed by centrifugation. Plasma volumes of between 5 and 10 cc were extracted 3 times with equal volumes of ether and the ether extracts were evaporated to dryness. The residue was dissolved in ethanol upon which ultraviolet analysis was carried out. The plasma extracts of 10 gravid women, 5 postmenopausal women and 14 immediately postpartum women were analyzed in this manner. To determine the recovery of progesterone added to blood plasma, human plasma was obtained from the blood bank and to it crystalline progesterone was added. The plasma thus prepared was extracted and analyzed and compared to control ethereal extracts of plasma. To determine the recovery of progesterone from the blood plasma of living animals, progesterone was administered intravenously to 9 rabbits. Three of this group had been subjected to total hepatectomy under ether anesthesia. Blood was withdrawn by cardiac puncture prior to the injection of the hormone as the control and blank. The progesterone was prepared for intravenous injection into the marginal ear vein of the rabbit in a microcrystalline suspension in 5 cc of normal saline. Cardiac blood was withdrawn at frequent intervals up to 30 minutes after injection of the progesterone suspension. This blood was extracted and analyzed as previously described. Two untreated rabbits were bled as



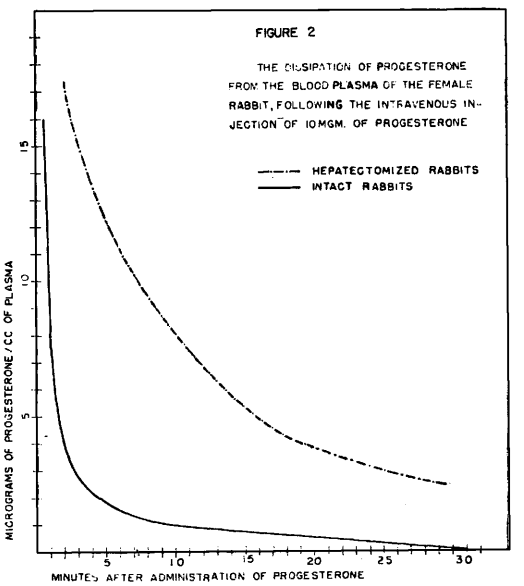


TABLE I.

Progesterone Plasma Levels, Expressed as μg of Progesterone per cc of Rabbit Plasma, Following the Intravenous Administration of Progesterone. Animals designated A are intact and B are hepatectomized.

	Minutes after the injection of progesterone												
	1/2	1	2	3	4	5	6	7	8	9	13	20	30
A-6	—	—	3.7	—	—	1.0	—	1.1	—	—	—	—	—
A-7	—	10.2	—	2.7	—	2.0	—	—	1.1	—	—	—	—
A-8	—	—	—	4.0	—	2.5	—	—	2.3	—	—	—	—
A-9	—	6.4	4.1	—	2.8	2.5	1.7	—	—	—	—	—	—
A-12	16.0	—	—	4.1	—	—	2.2	—	—	—	—	—	—
A-18	—	—	—	—	—	—	—	—	—	0.6	0.7	0.4	0.0
	—	—	—	—	—	—	—	—	—	—	—	—	—
Mean	16.0	8.3	3.9	3.3	2.8	2.0	2.0	1.1	1.7	0.6	0.7	0.4	0.0
B-10	—	—	14.8	—	—	15.0	—	—	—	—	—	4.4	—
B-15	—	—	20.0	—	—	—	—	—	—	—	—	3.8	2.2
B-17	—	—	—	—	—	—	—	—	8.8	—	—	—	—
	—	—	—	—	—	—	—	—	—	—	—	—	—
Mean	—	—	17.4	—	—	15.0	—	—	8.8	—	—	4.0	2.2

controls. One rabbit was bled for a period of 3 days following the intramuscular injection of 50 mg of progesterone in sesame oil. Hepatectomized and intact mature virgin rabbits were also given cholestenone and testosterone intravenously. One gravid woman with antepartum eclampsia was injected intravenously with 100 mg of progesterone dissolved in 5 cc of propylene glycol. Blood was obtained for analysis by venipuncture.

Results. The analysis of plasma from gravid, postpartum and postmenopausal women failed in any instance to indicate the presence of specific absorption at 240 mμ. Comparative analysis of the absorption curves obtained in these various physiological states revealed no common factor rendering them distinguishable as a group from either of the other groups. In general, the absorption patterns obtained were identical within each group and with those of the other groups.

Patient E. C., to whom 100 mg of progesterone was administered in propylene glycol, revealed no evidence of plasma progesterone prior to injection. At one minute after injection, ultraviolet analysis of the extracts of plasma revealed 3.8 μg/cc and at 8 minutes after injection, 1.6 μg per cc. The sodium pregnandiol glucoronidate urinary excretion was markedly increased two days after injection of progesterone. On the day of injection, the total urinary excretion was found to be 69 mg and 48 hours after injection was 122 mg. Sodium pregnandiol glucoronidate determinations were made after the method of Allen and Viergiver(10).

It was apparent that progesterone injected into the blood stream of the intact rabbits had an exceedingly short existence as ether extractable progesterone. Fig. 1 represents a typical example of the absorption patterns

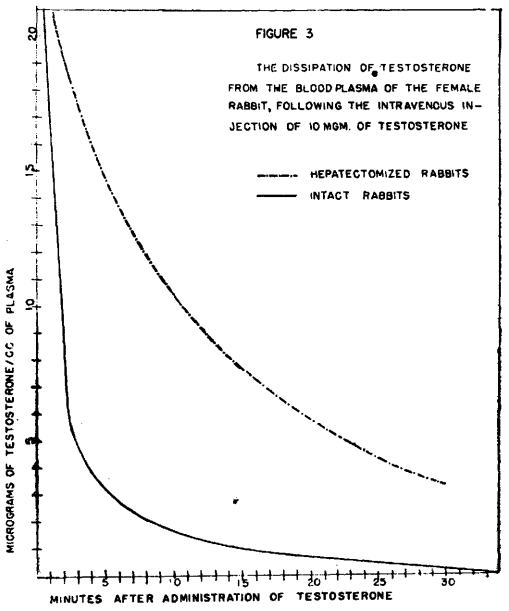


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TABLE II.

Testosterone Plasma Levels, Expressed as μg of Testosterone per cc of Rabbit Plasma, Following the Intravenous Administration of Testosterone. Animals designated A are intact and B are hepatectomized.

	Minutes after the injection of testosterone											
	1/2	1	2	3	4	7	8	10	15	16	25	30
A-19	20.0	—	—	3.6	—	—	—	—	—	—	—	0.0
A-21	—	—	5.2	—	—	2.5	—	2.2	0.9	—	—	—
A-22	21.3	14.4	—	7.5	—	—	—	2.2	—	—	—	1.5
	—	—	—	—	—	—	—	—	—	—	—	—
Mean	20.7	14.4	5.2	5.6	—	2.5	—	2.2	0.9	—	—	0.8
B-26	—	21.8	—	—	17.2	—	13.4	—	—	—	—	—
B-27	—	—	—	—	—	—	11.5	—	—	7.5	—	—
B-28	—	—	—	—	—	—	—	—	—	—	—	3.7
B-29	—	—	—	—	—	—	—	7.4	6.3	—	2.1	—
	—	—	—	—	—	—	—	—	—	—	—	—
Mean	—	21.8	—	—	17.2	—	12.5	7.4	6.3	7.5	2.1	3.7

obtained in the experimental animal prior to and after the administration of progesterone. The corrected curves were obtained by using the control analysis as the blank in the spectrophotometer. It will be noted that these corrected curves approach with constant accuracy the absorption pattern of crystalline progesterone dissolved in 95% ethanol. Fig. 2 and Table I illustrate the dissipation of progesterone from the blood plasma of 6 intact and 3 hepatectomized female rabbits. The progesterone level decreases rapidly in the first 6 minutes after injection and then more slowly until at 30 minutes after injection the steroid has disappeared. The hepatectomized group revealed a similar dissipation pattern except that the initial post injection decline was much less precipitous than in the intact animals. Plasma samples from the rabbit treated with intramuscular progesterone in oil at no time exhibited the presence of progesterone.

Those animals to which testosterone was administered, Fig. 3 and Table II, followed the same pattern of decline in circulating hormone level as the progesterone series in both the intact and hepatectomized groups. It will be noted that the initial levels obtained in the testosterone-treated animals were higher than the comparable progesterone-treated animals. This is attributed to the fact that testosterone has a higher solubility in aqueous solvents than does progesterone.

The third series of experimental animals comprise the group to which cholestenone was administered intravenously. At no time in either the intact or the hepatectomized group could a cholestenone plasma level be demonstrated. This was explained on the basis of the minimum solubility of cholestenone of less than 1 μg per cc of water at room temperature. The cholestenone was at no time in solution in the plasma and as particulate matter was rapidly removed from the plasma before even a circulating plasma suspension level could be attained. The recovery of progesterone from plasma to which it had been added was considered to be adequate and varied between 85 and 100%.

Summary. Ultraviolet absorption studies of ether extracts of the blood plasma of pregnant women failed to reveal the presence of progesterone. Progesterone was recovered from rabbit plasma by ether extraction and measured by ultraviolet analysis after the intravenous administration of the hormone. In a like manner, testosterone may be recovered from the plasma of rabbits after intravenous administration. Cholestenone plasma levels could not be demonstrated after the intravenous administration of this substance. Testosterone and progesterone have a short life as ether extractable substances in the plasma of intact female rabbits. Hepatectomy decreases significantly the rate of dissipation of ether extractable testosterone and progesterone from the blood plasma of the experimental animal.

Progesterone dissolved in propylene glycol and administered intravenously to a pregnant woman also had a short life as ether extractable steroid. This procedure was followed by a marked rise in urinary sodium pregnandiol glucoronidate excretion.

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Recovery of the Coxsackie Group of Viruses from Human Sources. (17708)

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In 1948 this laboratory reported(1) on the presence of neutralizing antibodies in human sera against the virus of Newcastle Disease (NDV). NDV was not isolated from any of the material examined, and it was shown later (2,3) that the positive neutralization tests were due to a nonspecific heat-labile factor in the sera. Since frozen specimens from the cases described in the previous paper were still available, following the report by Dalldorf and Sickles(4) on the recovery of the Coxsackie virus from human feces(5), this material was re-examined by inoculation into suckling mice. The Coxsackie virus was obtained from both the feces and nasopharyngeal washings of many of these patients. Subsequently, more specimens were examined from various sources, and virus strains have been recovered in material from 97 individuals in 9 different states: Alabama, Colorado, Delaware, Florida, Georgia, Louisiana, Oklahoma, South Dakota and Tennessee. This report presents the results thus far obtained in the study of the viruses isolated.

Methods. (a) *Treatment of feces or oral washings.* Fecal and oral specimens were prepared according to the method of Howitt and Barnett(6) for the recovery of poliomyelitis virus. Twenty percent suspensions of feces were made in distilled water. These were spun in an angle centrifuge at 13,000 r.p.m. for 30 minutes in the cold room. One thousand units of penicillin and 10 mg of streptomycin were added for each ml of the supernatant fluid, and the mixtures were left at room temperature for 30 minutes before inoculation of 3-to-5-day-old mice. Each animal received 0.01 ml intracerebrally, 0.03 ml intraperitoneally, and 0.03 ml intramuscularly of the treated feces. The oral washings were inoculated in the same manner, but the antibiotics were reduced to one-half the quantity. (b) *Virus isolations.* The fecal specimens received were kept frozen in the dry ice refrigerator while all other material was stored in the electric freezer at −10°C. The best results were obtained with oral specimens from patients who had gargled with 40 or 50 ml of tryptose phosphate broth. With specimens obtained early in the illness, the virus was found more often in the oral material than in the feces. The inoculation of 3-to-5-day-old mice with positive human material, produced symptoms of marked ataxia, followed by muscle paralysis progressing to complete immobilization, after which death might occur rapidly or be delayed for several days. Virus was present in the infected mice brains, but a larger amount could be harvested by skinning the animals and removing all skeletal muscles. The muscles were prepared as a 10% suspension in buffered saline contain-

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