cess of antiserum. Under these conditions, as might be expected, reactivation rarely occurs. In the mixture containing 1:10 virus we have the other extreme, namely, virus in excess. The injection of this material produced death even when diluted, merely confirming the potency of the virus.

The importance of having balanced mixtures of virus and antiserum in order to demonstrate reactivation by dilution is clearly brought out in the table. If antiserum is in excess, there is no detectable liberation of virus, and when virus is in excess the mixture is naturally potent. These findings agree with the work of Goyal[22] on vaccinia virus and possibly account for the negative results of Merrill. The extent of dilution is also a factor since the mixture containing virus in a concentration of 1:100 was not reactivated when further diluted 1:10 and 1:1000 but was reactivated in a dilution of 1:100.

Conclusions. 1. A neutral mixture of eastern equine encephalomyelitis virus and homologous antiserum was reactivated when a 1:100 concentration of virus with antiserum was diluted a hundredfold with 0.85% saline solution and was allowed to stand at 26°C (room temperature). 2. It appears that reactivation will occur only in a balanced mixture of virus and serum and furthermore, the extent of dilution is important.

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Effects of Repeated Small Doses of Roentgen Rays on Canine Blood and Bone Marrow.

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Since the human and experimental data on the effect of roentgen rays on the blood and the bone marrow deal with large doses and their sequelae over relatively short periods of time, 4 dogs were exposed to doses of 75 r or 100 r units administered at intervals of a week or longer over several months.

Before irradiation, dogs 11, 21 and 24 were given a course of subcutaneous benzol injections (preceded by 4% novocaine local

22 Goyal, G. K., *J. Immunol.*, 1935, **29**, 111.

anesthesia) over 12 weeks. This project was abandoned because of diffuse subcutaneous edema in dog 11 between the 8th and 12th weeks and in dog 24 between the 3rd and 8th weeks, and the development of draining sinuses in dog 21 between the 2nd and 8th weeks which had nearly completely healed at the 12th week.

The data for the benzol experiments are illustrated to contrast with those following irradiation which was given under nembutal anesthesia over the left side to overspread the caudal half of the left thorax and the cranial half of the left abdomen with the animal resting on its right side. A portal of 15 x 15 cm, a focal skin distance of 50 cm, 215 K.V., 15 m.a., filtration of ½ mm of Cu and 1 mm of Al, and 2½ min for 100 r were used. Venous blood samples were oxalated before each exposure for the counts recorded. The bone marrow was obtained by rib resection.[1]

In all animals the first line on the y-axis of the graph and row I in the table show the values noted before any experimentation was begun. In the graph, the arrows pointing up show individual rib resections which correspond to the bone marrow values presented in the table in chronological and Roman numerical order. The vertical lines indicate doses of benzol, 2 cc each for dogs 11 and 24 and 3 each for dog 21. The longer arrows pointing down mark doses of 100 r units of X-ray; the shorter arrows pointing down, 75 r units. The figures along the x-axis indicate weeks for benzol injections and days for X-ray exposures. In the table, N means normal; mark hype, markedly hyperplastic; mod hypo, moderately hypoplastic; C.I., cellularity indeterminable; hype, hyperplastic; and hypo, hypoplastic.

In dog 11 the total leucocytes in thousands per cu mm (WBC) were depressed, except for an occasional rise, at a level of 5,000 or slightly below. After 400 r units had been received, a marked rise in the percentage of stab neutrophiles (StN) and a proportionate decrease in the segmented neutrophiles were seen. The lymphocytes ranged generally from 12-26%, but occasionally dipped as low as 6%. The eosinophiles reached a few sharp peaks, the highest 17%. The monocytes, not plotted, ranged between 0-5%. During irradiation, metamyelocyte neutrophiles were intermittently present between 1-4%. Basophiles, never found in normal dogs,[2] occupied 1-2% of some counts. In these experiments they are believed to be neutrophiles inclosing basophilic degeneration granulations. Sporadically, a few of both the polymorpho- and mononuclear forms

[1] Mulligan, R. M., *Anat. Rec.*, 1941, **79**, 101.

[2] Mulligan, R. M., *Am. J. Physiol.*, 1941, **133**, 394.

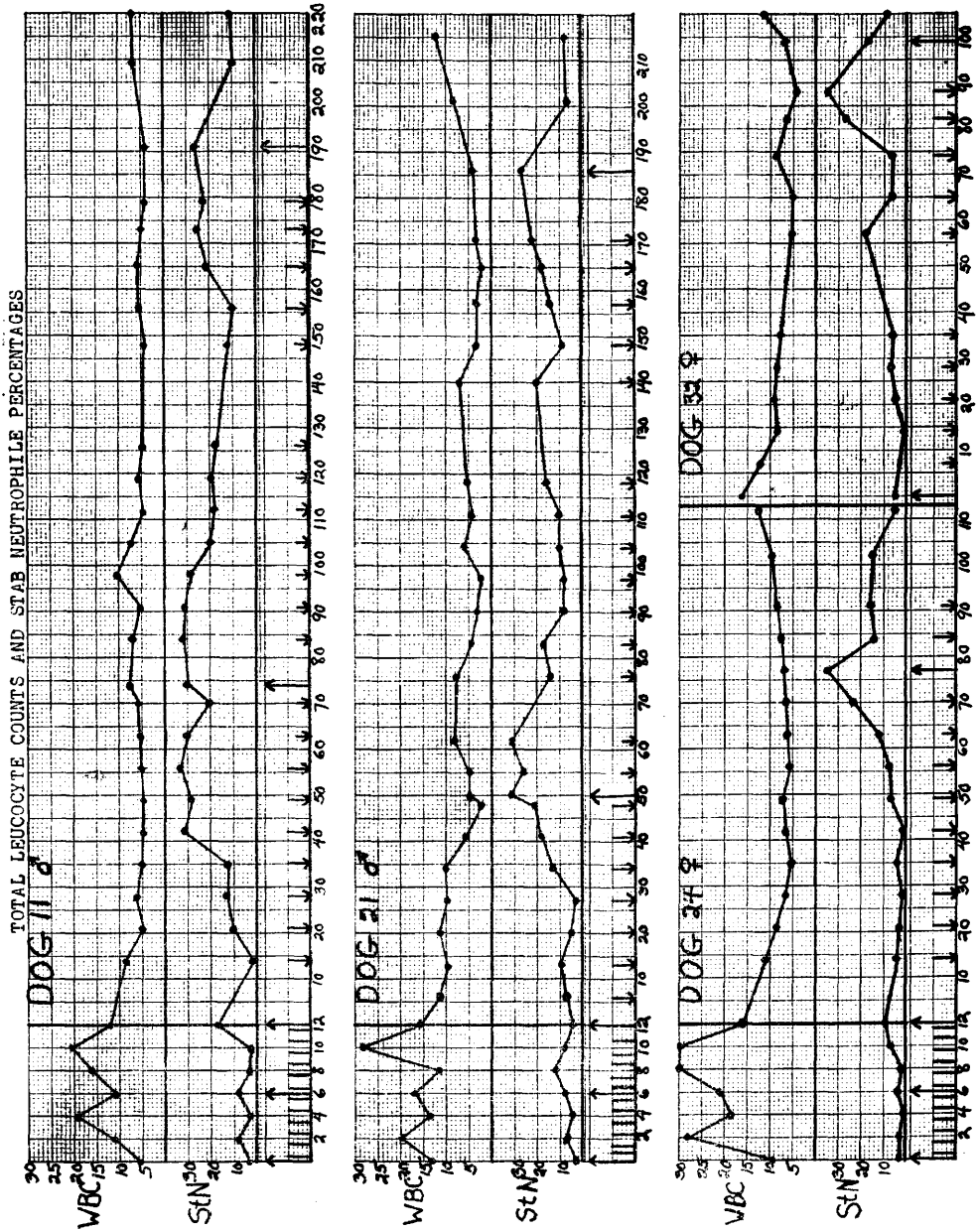

FIG. 1.

had bizarre nuclei and vacuolated cytoplasm. These and other damaged forms were not included in the counts. The erythrocytes were depressed from 5.5 to 4.5 million per cu mm after 850 r units. Corresponding changes in the hemoglobin (Newcomer) in grams

TABLE I.
Summary of Bone Marrow Studies.

	Eosinophiles	Promyelocyte neutrophiles	Myelocyte neutrophiles	Metamyelocyte neutrophiles	Stab neutrophiles	Segmented neutrophiles	Proerythroblasts	Erythroblasts	Normoblasts	Stem cells	Lymphocytes	Unidentified cells	Myeloid / Erythroid ratio	Cellularity
Dog No. 11														
I	4.6	2.0	4.6	5.6	24.6	4.0	0.8	1.6	46.4	1.4	0.8	3.6	0.9	N
II	3.0	2.2	4.0	13.0	33.4	2.2	0.2	0.6	37.0	0.6	1.6	1.8	1.5	N
III	2.0	1.8	3.8	17.4	26.4	1.8	0.8	1.4	42.0	0.4	0.8	1.4	1.2	mark hype
IV	2.0	0.8	2.0	6.6	11.0	0.6	0.6	1.8	70.0	0.2	2.2	2.2	0.3	mod hypo
V	2.4	0.4	3.0	7.6	16.0	1.0	0.2	2.6	61.2	0.0	3.6	2.0	0.5	mod hypo
Dog No. 21														
I	5.2	1.2	3.4	7.8	25.4	3.0	0.4	3.0	48.2	0.6	1.0	0.8	0.9	N
II	5.4	3.4	11.8	15.2	41.4	7.2	0.0	1.2	8.4	1.6	1.8	2.4	9.0	C.I.
III	2.0	1.4	7.0	18.2	35.4	4.4	0.0	0.8	26.2	0.2	2.0	2.4	2.5	mod hype
IV	2.4	0.2	1.8	9.4	23.0	1.0	0.0	2.2	56.4	0.0	2.0	1.6	0.6	hypo
V	2.6	0.0	4.0	11.4	22.0	1.2	0.4	3.4	52.2	0.0	1.2	1.6	0.7	hypo
Dog No. 24														
I	4.0	1.2	4.2	7.2	26.0	4.4	1.0	2.6	45.6	0.8	1.4	1.6	1.0	C.I.
II	4.4	2.4	7.8	14.2	30.8	3.2	0.0	0.2	29.2	0.8	3.8	3.2	2.1	hype
III	3.0	2.4	7.0	9.2	13.4	2.2	0.4	2.0	53.2	0.4	2.0	4.6	0.7	N
IV	3.0	1.4	3.6	9.6	18.2	2.4	0.8	1.8	53.8	0.6	2.8	2.0	0.7	mod hypo
Dog No. 32														
I	4.4	1.0	4.2	13.2	27.8	3.6	0.6	2.2	37.8	0.4	2.2	2.4	1.3	N
II	4.2	0.8	2.2	9.0	17.8	1.4	0.8	2.2	58.2	0.2	2.0	1.2	0.6	mod hypo

per 100 cc of whole blood were noted. Several times a high mean corpuscular hemoglobin was associated with hyperchromia of the erythrocytes. Normoblasts were irregularly noted up to 2%. The bone marrow after irradiation exhibited moderate hypoplasia and definite lowering of the myeloid/erythroid ratio.

At the same dosages (400 r and 850 r) dog 21 behaved essentially as dog 11, exhibiting leukopenia as low as 2,200, a lower average level of lymphocytes, 8-16%, and a drop in erythrocytes from 6.2 to 4.8 million per cu mm. The bone marrow of this animal after 700 r units was obviously hypoplastic. The myeloid/erythroid ratio of 9.0 after 6 weeks of benzol injections was undoubtedly due to the presence of the infected draining sinuses mentioned.

Dog 24 showed similar findings except for the absence of eosinophilia and of metamyelocyte neutrophiles in the blood. The lymphocytes ranged between 12-30%. The erythrocytes dropped from 6.4 to 4.6 million per cu mm after 950 r. After having received 1,025 r units, this animal was in heat, was mated with dog 11 on the 112th day of the experiment for each animal and delivered a normal litter of 2 male and one female puppies 58 days later. These nursed vigorously and are healthy at 4 months of age. The amount and timing of the X-ray exposure was not deleterious to the normal ovarian activity of this animal, since the ovaries lay directly in the path of the rays.

Dogs 11, 21, and 24 were irradiated on the same days until dog 24 was mated. Dog 32 was then substituted, received X-ray exposure on the same days as dogs 11 and 21, and demonstrated changes comparable to those seen in the other 3 animals. During the irradiation experiments, none of the animals evinced any disturbance whatsoever in appetite or weight; in the gastrointestinal tract; in the healing of wounds after rib resection; or in general activity. They were always fully recovered the day after anesthesia and X-ray exposure.

Summary. Repeated small doses of roentgen rays caused depression of the total leucocyte count, shift to the left in the neutrophile granulocyte line, occasional eosinophilia, and moderate anemia in the peripheral blood; hypoplasia and decrease in the myeloid-erythroid ratio in the bone marrow; and no evidence of interference with the general activity of the experimental animals.

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