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RADIOBIOLOGY

Factor in Serum inhibiting Bone Marrow Mitoses after X-irradiation of the Spleen

IT has been suggested that the spleen releases a mitosis inhibition after X-irradiation[1,2]. It has been found that after irradiation of the exteriorized spleen this inhibitor can be obtained by puncture from the congested spleen[3].

We have investigated whether the appearance of this inhibitor in the spleen is induced by irradiation alone or by the exteriorization and congestion of the organ. We also tried to detect the inhibitor in the circulating blood.

Rabbits were exposed in air to local irradiation of the spleen with doses of 400 r. (*THX*-250 apparatus, 180 kV, 10 m.amp, 0·5 mm copper, 30 cm full scale deflexion, 111 r./min).

3 h later, blood was withdrawn from the rabbits by heart puncture. After clotting, serum was separated by centrifugation and injected intramuscularly to rabbits weighing about 2 kg. The antimitotic effect was investigated by determining the reticulocyte number per 1,000 red blood cells. This was carried out in smears of blood collected from ear veins and stained with brilliant cresyl blue. At least 4–5,000, and sometimes as many as 8–10,000 red blood cells were counted in each smear.

The protein content of the injected serum was determined by the Lowry method.

The results in Table 1 show that intramuscular injection of the serum from rabbits with locally irradiated spleens depresses the number of reticulocytes in the blood. The reticulocyte number dropped to 50 per cent (sometimes even to less) of the value before injection of serum when the serum protein amounted to 70–100 mg protein/kg body weight.

Table 1

Serial number of rabbits: With irradiated spleen	Serial number of rabbits: Treated with serum	Intramuscular injection of serum protein: (mg/total)	Intramuscular injection of serum protein: (mg/kg)	Minimum reticulocyte number as percentage of number of reticulocytes before injection
107	108	280		54
	109	280		54
114	110	270		29
	111	243		43
115, 116	119	280		9
	120	280		62
135, 136, 137	125	224	100	42
	126	224	70	63
140	142	193	80	70
151	144	307	100	45
	145	240	100	65
	146	134	50	86

Table 2

Serial number of rabbits: Normal	Serial number of rabbits: Treated with serum	Injection of: Serum (ml./total)	Injection of: Protein (mg/total)	Injection of: Protein (mg/kg)	Minimum reticulocyte number as percentage of number of reticulocytes before injection
N 18	33	3			100
	34	3			100
N 21	24	4			100
	25	4, 5			100
N 149, 155	150	3, 8	227	90	82
	152	4	239	90	83
	153	2, 7	164	70	100

The reticulocyte number dropped to its lowest level 1–2 days after serum injection and returned to its original level, or sometimes to higher levels, in the next few days.

Serum from normal rabbits did not induce any drop in the reticulocyte number (Table 2). On the contrary, it was usually found to have risen 2–3 days after the injection of normal serum.

The isolation and characterization of the substance responsible for the mitotic inhibition will be described elsewhere.

To sum up, we have shown that after local X-irradiation of the spleen in lead shielded rabbits an antimitotic substance is released into the circulating blood and can be detected in the serum.

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Comparison of Calcium-48 and Carbon-14-proline as Indicators of Bone Metabolism

THE development of improved methods for the isolation and determination of hydroxyproline from bone[1] has made it possible to study collagen turnover *in vivo* in calcified tissues with a higher degree of accuracy than was previously attainable. This is accomplished by the administration of carbon-14-proline, followed by the isolation of hydroxyproline. The conversion of proline to hydroxyproline is a measure of collagen formation[2].

The use of the stable isotope calcium-48 as a tracer for bone mineral[3] offers a convenient method for studying simultaneously bone mineral turnover and collagen turnover. This report presents the results of a study in which bone metabolism was studied using calcium-48 and proline labelled with carbon-14 simultaneously. Experimentally produced tibial fractures in the rat were used as the stimulus for increased bone turnover.

Three adult female rats, weighing 220 g each, were anaesthetized and the right tibia was broken. After two weeks each rat was injected intraperitoneally with 25 μc. of uniformly labelled L-^{14}C-proline, specific activity 12·6 mc./mmole. Each animal was also simultaneously injected with 2 mg of calcium-48 in the form of calcium chloride. The rats were killed 24 h after the administration of the isotopes, and the femurs and tibias removed. The femurs were divided into proximal ends (20 per cent of the total length), shafts (60 per cent), and distal ends (20 per cent), and the tibias into proximal ends (20 per cent) and shafts plus distal ends (80 per cent).

The bones were demineralized for 3 days with 0·33 normal hydrochloric acid, and the organic residue was then refluxed with 5 ml. of water using a sand bath and an air condenser[4]. The undissolved organic matter was dispersed into small particles by vigorous mixing, and