Though himself gaining distinction in the biochemical world, Raper looked askance at the modern trends which have so widely substituted biochemistry for chemical physiology. He felt that the future of scientific medical practice was endangered by the threat of divorce between biochemistry and physiology, and the strength of his conviction may perhaps partly explain Manchester's reluctance to follow the lead given by other universities towards the establishment of an independent biochemical school. His chief delight was in the laboratory, where he preferred to work himself rather than to direct others. Regretfully, as the years passed, he found the time available for the laboratory severely curtailed, and after the fruitful collaboration with Harper which in 1943 resulted in the identification of a new hormone, pancreozymin, he found himself fully occupied with teaching and administrative duties. Complete honesty and a genial sympathy with his fellow men, abundance of tolerance and tact, these were in him outstanding qualities. They were coupled with a remarkable tenacity of purpose when his considered judgement had been reached. With such qualities

it was inevitable that his advice should be widely sought and that his responsibilities should be enlarged. In 1946, reluctantly, he allowed himself to be persuaded to vacate the chair of physiology and to devote himself entirely to the Deanship of the Medical School, and to the host of ancillary duties devolving upon the holder of that office. Manchester will long remain in his debt for his unselfish devotion to the cause of medical education in these last years. The condition of Raper's heart had long been a cause of anxiety to his colleagues and friends, yet he carried on in full harness and with undiminished effectiveness for nearly five years after he had reached the usual retiring age. His impending retirement at Christmas 1951 would have been well-earned, and he was looking forward with quiet pleasure to peaceful days in his garden or at his easel in the countryside. It seemed a cruel fate that, by his unexpected death on 12 December, denied these to him.

L. P. KENDAL

The photograph of the late Professor H. S. Raper is reproduced by kind permission of the Editor, *Medical School Gazette*, the University of Manchester.

Some Aspects of Phosphorus Metabolism in Bone Marrow

2. CHANGES IN THE CONTENT OF PHOSPHORUS COMPOUNDS AND REDUCING SUBSTANCES IN BONE MARROW AND SPLEEN, CAUSED BY IONIZING RADIATION AND OTHER FACTORS WHICH DEPRESS THE FUNCTION OF BLOOD-FORMING TISSUE

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The experiments reported below are a continuation of studies described in an earlier paper (Lutwak-Mann, 1951); the investigation has been extended to provide further information about the behaviour of nucleic acid phosphorus, lipid phosphorus, ascorbic acid and glutathione in rat bone marrow and spleen, under various experimental conditions including dietary changes, total body exposure of normal and splenectomized animals to different single doses of X-rays, thermal skin burns and the action of certain myelotoxic agents and mitotic inhibitors.

MATERIAL AND METHODS

Material. Unless otherwise stated, male rats weighing 180–250 g. were used; the bone marrow collection from the long bones was carried out as previously described (Lutwak-Mann, 1951). Based on the experience gained in earlier studies, an effort was made to use homogeneous groups of

animals for any given experimental series, so as to minimize the differences in their response to pharmacodynamic agents.

Methods. The determinations of acid-insoluble P and the abbreviations for the P fractions were the same as before (Lutwak-Mann, 1951). Ascorbic acid was determined by the method of Roe & Kuether (1943), glutathione according to Hopkins & Morgan (1936) and ergothioneine by the method of Hunter (1951).

The effects of dietary changes on the P composition of bone marrow were studied in rats maintained from the age of 6 weeks on the following carbohydrate-free, high-fat diet: arachis oil 75 parts, light white casein 20, salts 5, together with 12 parts of Marmite, either untreated or autoclaved, and the usual daily supplement of cod-liver oil; the animals which received autoclaved Marmite were given parenterally 50 µg. thiamine daily. When transferred to this diet, growth continued for 1 month and thereafter ceased, irrespective of the addition of thiamine; the condition of the coat in these rats was very poor, but the animals ate well and, except for the arrest of growth, showed no other symptoms. Some of

the animals were used at the age of 3.5 months, when their weight did not exceed 100 g., while others were transferred to a full stock diet for a further period of 2 or 4 weeks, when their weights increased by 50 and 85 g., respectively; simultaneously, there was a spectacular improvement in the condition of their coats. Some experiments were also carried out with male rats castrated at the age of 4 weeks and maintained from the third month for a 4-week period either on a full synthetic or on a vitamin B-deficient diet, made up as before (Lutwak-Mann, 1951).

Irradiation experiments were carried out at the Department of Radiotherapeutics, by kind permission of Prof. J. S. Mitchell. Total exposure to single doses of 300, 500, 600 and 800 r. was studied, the physical constants being the same as described earlier. Different batches of rats varied in their susceptibility, particularly to doses above 500 r., not only in the clinical sense, but also in the rate of recovery of the bone marrow P components. Thus for instance, with doses up to 600 r. no haemorrhagic symptoms or deaths were encountered during the 10–14-day routine experimental period, with young adult males of the laboratory stock. For some of the 600 r. irradiation experiments, however, 6–8 month-old female rats were used; several of these developed haemorrhages on the 5th–8th day after irradiation and 30% died within the first 2 weeks. With laboratory stock rats and a dose of 600 r., at least 50% recovery in the bone-marrow nucleic acid P content took place by the 7th day after exposure; but in rats from other sources, sometimes a lower and sometimes a more advanced degree of recovery was found at that time. A dose of 800 r. was lethal to over 75% of rats of all strains, but few succumbed before the 4th day after exposure.

Splenectomies and mock splenectomies were carried out by Dr Pamela Holton at the Department of Physiology, Cambridge; the blood of the operated animals was examined for *Bartonella muris* by Dr P. Tate, Molteno Institute, Cambridge. The incidence of bartonellosis after the removal of the spleen varied greatly; in one series of twenty-seven albino rats none developed the infection throughout a period of several weeks; in the rats of the laboratory stock about 30% succumbed at various times, mostly a few days after operation but sometimes much later. It was possible to prevent the occurrence of early bartonellosis by daily injections of neoarsphenamine; however, in several rats treated in this manner a late macrocytic anaemia developed, although the blood smears were negative for *Bartonella* inclusion bodies. It may be added here that on two occasions acute bartonellosis developed in non-splenectomized rats a few days after exposure to 300 r. X-rays.

Thermal skin burns were produced in the following manner. An area of skin on the back of the animals 2 in. long and 1 in. wide was freed of hair; 2 days later the experiment was carried out under ether-anaesthesia, by firmly pressing to the skin for 30 sec., calico bags of suitable size, filled with 20 g. of dry sand, preheated so as to maintain a temperature of 75°. Superficial and fairly uniform skin burns were produced in this way. Altogether 35 male rats weighing 200–250 g. were used for these experiments. After the heat treatment the animals were placed in individual cages to prevent contamination of the skin. A dry scab usually formed by the 4th–6th day; hair began to regrow from the end of the second week onwards. There was no evidence of discomfort or restriction of movement and the rats ate well and did not lose weight. The routine experimental period did

not exceed 2 weeks, but one group of six rats survived a month without a single casualty.

Aminopterin (4-aminofolic acid) and amethopterin (4-amino- N^{10} -methylfolic acid) were made available through the courtesy of Lederle Laboratories Division, American Cyanamid Co., New York, and were given by intraperitoneal injection in single or multiple doses. Colchicine, amidopyrine, and mustard sulphoxide together with dimethyldithiocarbamate, were injected subcutaneously. Muscle adenylic acid, yeast adenylic acid and inosinic acid were injected intraperitoneally as Na salts; only the first two were active pharmacologically in producing within about 1 min. after administration a 'shock'-like condition in the rats, which, however, wore off after 30 min. Yeast adenylic acid appeared more active than muscle adenylic acid. Different batches of rats varied in the extent of their response to these two nucleotides.

RESULTS

Lipid phosphorus content in bone marrow and spleen

The content of lipid P in the bone marrow of rabbits and rats has been determined previously (Lutwak-Mann, 1951); many more determinations have now been carried out both in rat bone marrow and in spleen. Relatively little variation was found, the bone marrow containing 50–60 mg./100 g. (as against 10 mg./100 g. in rat blood), while the spleen contained 60–70 mg./100 g. Bone marrow was found to contain enzymes which cause the breakdown of lipid P; when an aqueous suspension of bone marrow was incubated at 37° for 1 hr., the content of lipid P fell slightly and after a further 3–4 hr. (in the presence of toluene) less than one-third of the original amount remained. Rat spleen behaved similarly.

Ascorbic acid, glutathione and ergothioneine in bone marrow and spleen

Earlier determinations have established the content of glutathione and ascorbic acid in rabbit bone marrow (Lutwak-Mann, 1949). Numerous analyses were now carried out to determine ascorbic acid in rat bone marrow. In fresh tissue an average content of 24 mg./100 g. was found, estimated as oxidized ascorbic acid (Roe & Kuether, 1943); estimations carried out by means of the 2:6-dichlorophenolindophenol titration gave very similar values. Although there is a delay in the collection of bone marrow, it is unlikely that this could affect significantly the level of ascorbic acid, as it was found that small amounts of ascorbate well mixed into bone marrow and left at room temperature for 15 min. could be quantitatively recovered. The average content in rat spleen was 36 mg./100 g. However, in some batches of rats as much as 51 mg. ascorbic acid/100 g. was found in the spleen.

For the iodometric assay (Hopkins & Morgan, 1936) of glutathione in bone marrow, material from six to eight rats was pooled; fairly satisfactory agreement was found in three such series, the values being 198, 150 and 165 mg./100 g. Rat spleen contained 165–180 mg. glutathione/100 g.

Ergothioneine was determined in 1.4 g. rat bone marrow which was found to contain 2.7 mg./100 g.; rat spleen contained less than 1.0 mg./100 g.

The effect of dietary changes on the phosphorus composition of bone marrow

Previous studies were concerned with the effect upon bone marrow of quantitative restriction of normal food and of food deficient in vitamin B. In the present series of experiments the animals were maintained on a high-fat diet which had an adequate calorie as well as protein content, and which was not deficient in vitamins; it was, however, poorly balanced owing to the excess of one constituent, liquid arachis oil, and it failed to support normal growth, regardless of whether the animals received untreated Marmite, or autoclaved Marmite plus a daily supplement of 50 μ g. thiamine. Table 1

vitamin B-deficient diet, the same changes occurred in their bone marrow as had previously been described for non-castrate rats (Lutwak-Mann, 1951). Testosterone propionate, while it had its usual activating effect upon the accessory organs of the vitamin B-deficient castrates, did not influence the bone marrow composition in any significant manner.

The effect of total body exposure to different doses of röntgen radiation on the content of phosphorus, ascorbic acid and glutathione in bone marrow and spleen

Following the exposure to 300, 500, 600 and 800 r., the bone marrow and spleen were analysed (a) within a few minutes after irradiation, (b) 6 hr. later and (c) 2, 7 and 10 days later, for their content of nucleic acid and lipid P, ascorbic acid and, in some experiments, glutathione. The changes observed in the content of nucleic acid and lipid P in the bone marrow of laboratory stock young adult male rats exposed to 300, 600 and 800 r., are seen in Fig. 1. Except with the highest dose employed, it was not possible to register the changes which

Table 1. *Nucleic acid and lipid phosphorus content in the bone marrow of male rats, maintained on a high-fat diet*

(Groups 1–3 contained four rats each; group 4 contained two rats. Values given are mean and range.)

Group no.	Diet	(mg. P/100 g.)		
		DNA	RNA	Lipid
1	High-fat diet from the age of 6 weeks to 3.5 months	86 (78–93)	50 (48–52)	37 (31–44)
2	Same period of high-fat diet as in group 1, followed by 2 weeks of full stock diet	120 (110–132)	66 (59–77)	44 (42–46)
3	Same period of high-fat diet as in group 1, followed by 4 weeks of full stock diet	155 (148–163)	75 (71–77)	58 (58–59)
4	Full stock diet for 4 months	168 (166, 170)	73 (72, 73)	58 (56, 60)

gives the P distribution in the bone marrow of rats at the age of 3.5 months, that is, after 8 weeks of the high-fat diet; it is clear that considerable changes have taken place not only in nucleic acid but also in lipid P. However, it was found that the damage was not permanent, because when some of these animals were transferred to a full stock diet for relatively short periods of 2–4 weeks, considerable improvement in the chemical composition of the bone marrow was noted, parallel with the resumption of growth.

Here may be mentioned an experiment with forty male rats castrated at the age of 4 weeks, and kept thereafter on a full stock diet until they reached the age of 3–4 months. It was found that the bone marrow of the castrates did not differ in its P pattern from that of non-castrate male rats. When the castrated animals were placed for 1 month on a

presumably occurred immediately after irradiation, by means of the chemical methods used in this study. However, after 6 hr. there was already a pronounced breakdown of nucleic acid P, even with the relatively small dose of 300 r., whereas lipid P remained practically unaltered. Two days after exposure to 800 r., the composition of the material removed from the long bones resembled blood, rather than bone marrow; a considerable degree of loss in nucleic acid and lipid P was also caused by 600 r.; 300 r. did not affect lipid P very much, but nucleic acid P was well below half its initial value. At 7 days, there was marked recovery after the smallest dose; the majority of the rats irradiated with 600 r. showed at least a 50 % recovery at that stage and in those which survived 800 r. there was a trend towards recovery. Animals were not examined at 10 days following exposure to 800 r.;

those which had received 300 r. were almost fully recovered while those exposed to 600 r. also showed considerable improvement over the preceding stage.

large doses of radiation, the loss in nucleic acid P content seldom amounted in the spleen to more than 30 or 40 % of the control value. Recovery also appeared more rapidly than in the bone marrow,

Fig. 1. The behaviour of nucleic acid and lipid P in the bone marrow of rats exposed to 300, 600 and 800 r. X-radiation. Blank areas represent lipid, shaded areas nucleic acid P.

Table 2. *Effect of X-rays on the content of nucleic acid and lipid phosphorus, and ascorbic acid in bone marrow and spleen*

(Each value is the average for three rats. Exp. 1 was carried out with male albino rats; Exp. 2 with male hooded rats.)

Exp. no.	Dose of radiation (r.)	Time after exposure to X-rays	Nucleic acid (mg. P/100 g.)		Lipid (mg. P/100 g.)		Ascorbic acid (mg./100 g.)	
			Bone marrow	Spleen	Bone marrow	Spleen	Bone marrow	Spleen
1	0	—	233	152	58	62	26	48
	300	6 hr.	137	137	50	59	18	—
	300	2 days	128	110	48	54	12	38
	300	7 days	159	125	60	60	22	42
	0	—	248	128	55	69	23	36
	800	6 hr.	78	97	39	60	12	31
	800	2 days	12	79	24	52	7	26
	800	7 days	100	112	33	—	15	35

With regard to the behaviour of the spleen in the irradiated animals, the following observations were made; the changes in nucleic acid and lipid P content were less pronounced than those in the bone marrow, with exposure to radiation up to 500 r.; above this amount, there was a distinct fall in the content of nucleic acid P, at 2–4 days after irradiation, but lipid P remained relatively unchanged. Even with

but it was difficult to assess its extent owing to the spectacular changes in weight which rat spleen undergoes in response to radiation, so that while the concentrations might return to the pre-irradiation level, the amount per organ might be considerably less than in untreated animals. In Table 2 results are given for bone marrow and spleen following exposure to 300 and 800 r.

Table 2 also contains data for the amount of ascorbic acid in these two tissues. It can be seen that 50 % or more of the original content of ascorbic acid was lost from the bone marrow at the post-irradiation stage corresponding to the maximum breakdown of nucleic acid P; there was also a decrease in spleen but to a much smaller extent. The recovery in the P content during the later period was always accompanied by a return of the level of ascorbic acid to near-normal values.

Table 3. *Content of glutathione in bone marrow and spleen of rats, 2 and 7 days after total body exposure to 500 r. X-rays*

(Material from six to eight rats was pooled for the determination of glutathione.)

Group of rats	Glutathione (mg./100 g.)			
	Bone marrow		Spleen	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2
Non-irradiated	198	150	165	172
2 days after irradiation	84	110	115	130
7 days after irradiation	155	128	160	148

The iodometric assay of glutathione required large quantities of rat bone marrow, and only a limited number of irradiation experiments were carried out in which the content of glutathione was determined at 2 and 7 days after an exposure to 500 r. The values recorded in Table 3 indicate that the amount of glutathione decreased in the bone marrow of the irradiated animals 2 days after exposure while at 7 days the values were not much below the controls. Results with spleen were less clear; in one experiment the trend was analogous to that in bone marrow, but in the other the change appeared insignificant. It is possible that other animals may react more vigorously in this respect and that more profound changes might be observed in the content of glutathione with larger or repeated doses of radiation.

The effect of muscle adenylic acid, yeast adenylic acid and inosinic acid on the rate of nucleic acid phosphorus recovery in the bone marrow and spleen of irradiated rats

It was stated above that laboratory stock rats exposed to 600 r. radiation consistently showed, at 7 days, at least 50 % recovery in their content of nucleic acid P in the bone marrow, and nearly normal values in the spleen. An attempt was made to influence the rate of recovery in the following manner.

Three series of rats each containing fourteen to eighteen animals were used; four animals in each series were used as controls while the rest were injected daily with 15 μ g./g. of muscle adenylic acid, yeast adenylic acid or inosinic acid, for 4 days before irradiation. On the day of irradiation, half of this group were given an injection immediately before exposure, and thereafter for 5 consecutive days, whereas the

remainder, including the untreated controls, were irradiated but did not receive any further injections. All animals were examined on the 7th day after irradiation. At autopsy it was found that the rats which had received muscle or yeast adenylic acid both before and after irradiation, but not those treated with inosinic acid, had a bone marrow which in its gross appearance resembled an early post-irradiation period, in that it was fluid and contained blood, while in animals which had either not been injected at all or had only been injected before the exposure, the bone marrow was already of almost normal firm consistency, without blood

being present. The only gross change in the spleen was in weight: it was larger (580–650 mg.) in the group treated before and after irradiation with muscle or yeast adenylic acid than in the rest (380–480 mg.).

The quantitative results concerning P composition, as well as ascorbic acid, are given in Table 4. It can be seen that the continuous treatment with muscle or yeast adenylic acid distinctly retarded the recovery of the bone marrow and spleen, as judged by the level of the substances determined in these experiments; inosinic acid had no comparable effect. Administration of either of the two active nucleotides before irradiation only, appeared to have either no effect on the rate of recovery, or only a slight one, in some rats pre-treated with yeast adenylic acid. It must be added here that in separate experiments it was ascertained that eight consecutive injections of muscle or yeast adenylic acid, not followed by irradiation, had no significant effect upon the P content of rat bone marrow.

For the present, the author has no opportunity to extend these experiments sufficiently to assess fully their significance; they are, however, quoted both for their inherent biological interest as well as to underline the usefulness of quantitative chemical assays in the examination of haemopoietic tissue.

Effect of splenectomy on the phosphorus pattern of bone marrow

These experiments have been carried out in collaboration with Dr Pamela Holton, who splenectomized fifty rats. In one-third of the animals the content of nucleic acid and lipid P was determined at various stages between 2 weeks and 2 months after the operation; the remainder of the splen-

ectomized animals were used for irradiation experiments, in which they were exposed to single doses of 300 and 500 r. of radiation, at 1, 2, 3, 6 and 7 weeks after splenectomy.

It was found that the bone marrow of the splenectomized animals showed the same P pattern as that established for normal animals. On several occasions the bone marrow was examined in rats which as the result of splenectomy suffered from acute bartonellosis; in these the ribonucleic acid (RNA) value in the bone marrow was distinctly

The effect of skin burns on rat bone marrow and spleen

To determine whether substances released from burned skin are capable of altering the chemical composition of bone marrow or spleen, experiments were carried out in which adult male rats were examined at various stages after the exposure of a limited area of the skin to 75° for 30 sec. Analyses of bone marrow and spleen failed to produce evidence of any significant changes in these tissues.

Table 4. *Effect of treatment with muscle adenylic acid, yeast adenylic acid and inosinic acid on the bone marrow and spleen of rats irradiated with X-rays (600 r.) and examined 7 days after irradiation*

(The amount of nucleotide injected daily was 15 µg./g. The values represent averages; range in brackets.)

Exp. no.	Nucleotide injected	Daily doses		Rat no.	Bone marrow			Spleen		
		Before irradiation	After irradiation		DNA (mg. P/100 g.)	RNA (mg. P/100 g.)	Ascorbic acid (mg./100 g.)	DNA (mg. P/100 g.)	RNA (mg. P/100 g.)	Ascorbic acid (mg./100 g.)
1	None	0	0	4	102 (90-116)	67 (62-69)	11 (10-12)	64 (55-79)	60 (53-61)	29 (26-34)
	Muscle adenylic acid	4	0	7	89 (61-102)	62 (58-67)	11 (10-13)	66 (57-69)	66 (58-69)	—
		4	5*	7	28 (16-47)	37 (24-40)	6 (4-8)	45 (37-54)	49 (40-56)	19 (17-22)
2	None	0	0	4	116 (88-132)	68 (60-71)	12 (9-15)	68 (60-72)	63 (58-68)	34 (29-37)
	Yeast adenylic acid	4	0	6	88 (59-97)	57 (40-69)	11 (8-12)	55 (52-65)	62 (58-68)	—
		4	5*	7	23 (6-39)	24 (11-49)	6 (4-8)	44 (39-49)	53 (42-56)	22 (18-25)
3	None	0	0	4	111 (89-122)	66 (59-71)	11 (9-12)	66 (57-70)	59 (51-63)	31 (29-36)
	Inosinic acid	4	0	4	115 (92-124)	68 (61-76)	11 (9-13)	—	—	—
		4	5	6	121 (98-129)	70 (60-75)	14 (11-16)	79 (56-82)	63 (49-68)	33 (21-37)

* Bone marrow fluid, with considerable admixture of blood.

higher than the highest recorded for normal rats, i.e. 110 mg./100 g. against 85-95 mg./100 g. No changes were observed, however, in the content of deoxyribonucleic acid (DNA) or lipid P in the bone marrow. The only other instance where similarly elevated RNA values were observed was in the bone marrow of rats infected with *Trypanosoma lewisi* (placed at the author's disposal by Dr J. Ryley).

As regards the effect of irradiation on splenectomized rats, here too the changes observed in the bone marrow were indistinguishable from those found in the control unoperated or mock-splenectomized rats, irradiated simultaneously with the splenectomized animals.

The effect of aminopterin, amethopterin, colchicine and amidopyrine on the content of phosphorus and ascorbic acid in bone marrow and spleen

The previous study (Lutwak-Mann, 1951) demonstrated the striking effect upon the nucleic acid P content in the bone marrow of rats suffering from systemic poisoning with mustard gas or mustard sulphoxide combined with dimethyldithiocarbamate. It was of interest to investigate the effect of other myelotoxic agents and to compare their action with that of ionizing radiation.

Table 5 gives the results obtained with parenteral doses of aminopterin ranging from 0.18 to 0.5 µg./g.

Table 5. *Effect of aminopterin, amethopterin, mustard sulphoxide + dimethyldithiocarbamate, and colchicine on the content of nucleic acid P, lipid P and ascorbic acid in rat bone marrow and spleen*

(Each value represents the average for two to three rats. Male rats used in Exp. 1; female rats in Exp. 2.)

Exp. no.	Substance injected	Daily dose (no.) (μg./g.)		Interval after last dose (hr.)	Condition of animals	Gross appearance of bone marrow	Bone marrow				Spleen			
							DNA (mg. P/100 g.)	RNA (mg. P/100 g.)	Lipid (mg. P/100 g.)	Ascorbic acid (mg./100 g.)	DNA (mg. P/100 g.)	RNA (mg. P/100 g.)	Lipid (mg. P/100 g.)	Ascorbic acid (mg./100 g.)
1	None	—	—	—	Good	Normal (coral pink, firm)	152	80	60	26	107	65	68	51
	Aminopterin	7	0.18	1	Fair	Very pale, rather firm	82	43	58	20	86	54	60	52
		8	0.25	24	Fair	Thin, blood admixture	68	39	40	18	94	50	—	50
		1	1.70	5	Good	Normal	128	70	57	18	104	58	58	48
		2	1.50	120	Moribund	Very thin, large admixture of blood	35	20	25	7	92	53	—	39
		2	0.50	168	Poor	Colourless, firm	47	55	40	10	89	59	—	45
	Amethopterin	4	1.60	24	Fair	Speckled, fatty	145	74	53	16	101	56	—	49
		6	1.60	24	Fair	As above	94	46	52	13	109	50	58	50
	Mustard sulphoxide + dimethyl dithiocarbamate	1	20 + 100	20	Fair	Very thin, large admixture of blood	55	70	43	12	79	51	—	38
		1	20 + 100	40	Poor	As above	25	26	40	9	66	46	52	40
2	None	—	—	—	Good	Normal	162	88	52	20	132	66	66	32
	Colchicine	1	2.00	8	Poor	Dark red, jelly-like	106	52	42	10	124	55	61	27
		1	2.00	24	Poor	As above	72	32	48	5	111	46	50	26
		1	15.00	24	Moribund*	As above	65	21	34	8	117	54	57	25

* Spleen weight 280 mg. as compared with average weight of 800 mg. in the normal rats.

body weight, at different stages of the treatment. Rats appeared capable of withstanding multiple small doses of aminopterin which, though they did not provoke clinical manifestations, nevertheless led to a considerable depression in the level of nucleic acid P in the bone marrow without, however, affecting the spleen. A single large dose of aminopterin did not produce obvious changes within 5 hr.; but two such doses proved lethal if the animals were left sufficiently long for the general symptoms to develop; again, it was found that the bone marrow was selectively affected. A striking feature, not observed after other treatments, e.g. X-radiation, was the gross appearance of the bone marrow in the later stages of aminopterin treatment, when it was almost colourless, although of almost normal firm consistency. Lipid P was little changed after small doses, but fell significantly after the administration of larger amounts. Loss in the content of ascorbic acid accompanied the decrease in P content of the bone marrow.

Amethopterin was much less toxic to rats than aminopterin; its effects were also confined to the bone marrow (Table 5).

A single dose of colchicine ($2 \mu\text{g./g.}$ body weight) produced a considerable depression in nucleic acid P of the bone marrow 8 hr. after injection; a further decrease was noted at 24 hr. Colchicine appeared to affect the RNA fraction even more severely than DNA, especially in the experiment with a lethal dose of $15 \mu\text{g./g.}$ The effect on ascorbic acid was pronounced, but on lipid P it was relatively slight. Rat spleen reacted to colchicine treatment by a fall in weight sometimes to one-third of the control value, but the chemical changes were not striking.

Table 5 records also some experiments with mustard sulphoxide plus dimethyldithiocarbamate; the effect of these two substances on the bone marrow P has been demonstrated earlier; it was found now that, as with X-rays, it was not limited to the bone marrow but extended also to the spleen. There was also a decrease in the content of ascorbic acid, more pronounced in the bone marrow than in spleen.

On the other hand, amidopyrine, which is known to inhibit rat bone marrow glycolysis *in vitro* (Lutwak-Mann, 1949), failed to produce changes in the bone marrow or spleen, even after prolonged treatment with amounts up to 0.1 mg./g. daily.

DISCUSSION

The findings made in the course of this study have demonstrated a difference in the susceptibility of important tissue constituents to the impact of certain physical and chemical agents applied to the animal as a whole. Thus, with suitably graded doses of röntgen radiation, it was possible to produce a major breakdown of nucleic acid in the bone marrow

and spleen, without affecting the content of lipid P to any marked extent. In general, the reactivity of the nucleic acid P towards a variety of agents has proved to be much greater than that of the lipid P; the changes in nucleic acid P were accompanied in all instances by a decline in the content of ascorbic acid and presumably also of glutathione, though with regard to the latter, a further strengthening of the experimental evidence is desirable.

In this connexion it may be recalled that earlier attempts to correlate the glutathione and ascorbic acid content of organs such as liver with the extent of the X-ray injury were inconclusive (Lutwak-Mann, 1951), although marked changes in the content of these reducing substances do occur in the liver in response to pharmacologically active agents, such as salicylates and similar aromatic substances (Lutwak-Mann, 1942). Nevertheless, the depletion in ascorbic acid of the bone marrow and to a lesser extent of the spleen may be taken to be established as a result not only of irradiation, but also of the action of chemically unrelated substances, such as mustard gas, aminopterin and colchicine.

It is interesting to record in blood-forming tissue, in addition to ascorbic acid and glutathione, the presence of yet another reducing substance, ergothioneine, which has also been found in red blood corpuscles (Hunter, 1951) and in the reproductive tract (Leone & Mann, 1951).

The comparative study of bone marrow and spleen has indicated a qualitative similarity of their behaviour in most, if not in all, experimental conditions: whereas exposure to X-rays or to mustard sulphoxide induced analogous, though quantitatively different, chemical changes, the folic acid antagonists and, to a smaller extent, colchicine appeared to act selectively on the bone marrow. However, before attempting to evaluate the effect of any given agent on spleen, in relation to the whole animal, account must be taken of the conspicuous shrinkage of this organ at certain stages of the experimental period.

By selecting homogeneous groups of experimental animals, it was possible to establish satisfactorily the extent of nucleic acid P recovery in bone marrow and spleen, at an arbitrarily chosen stage, 7 days, following exposure to 600 r. X-rays. An attempt was then made to influence this process by subjecting the animals to treatment with muscle adenylic acid or yeast adenylic acid, before and after irradiation. Both the gross appearance and the chemical analyses, particularly of bone marrow, but also of spleen, at 7 days after irradiation, pointed to an interference with the recovery of these tissues in the nucleotide-treated animals. While much further work is necessary to assess the significance of these results, it is instructive to find that only the nucleotides with an intact adenine-

amino group appeared active, inosinic acid being devoid of potency in this respect. It is known from earlier researches, notably those of Bennet & Drury (1931), that muscle adenylic acid and yeast adenylic acid, administered intraperitoneally, produce leucopenia followed by leucocytosis. It therefore seems not unlikely that prolonged stimulation of the blood-forming tissue by suitably timed treatment with these nucleotides may, at any rate in a certain proportion of animals, modify the response to ionizing radiation and possibly to other myelotoxic agents as well.

A further instance of the deleterious effect which a poorly balanced diet may exert upon the bone marrow was provided by experiments with the high-fat diet which, although not deficient in vitamins or protein, failed to promote growth and led to a remarkable diminution in the content not only of nucleic acid but also of lipid P. On the other hand, gonadectomy, splenectomy or mild-degree skin burns, produced no major change in the P pattern of rat bone marrow. As with all such work, the possibility remains that other animal species would react differently under such circumstances, especially perhaps as regards X-ray injury after the removal of the spleen.

Finally, it may be said that, while it is not likely that the quantitative chemical approach introduced in this study could supersede the well established cytological methods for the study of haemopoietic tissue, it nevertheless must be reckoned as a most useful subsidiary, capable of great development.

SUMMARY

1. The behaviour of acid-insoluble phosphorus and of ascorbic acid and glutathione in rat bone marrow and spleen has been studied under experimental conditions which included dietary changes, X-ray injury, splenectomy, thermal burns and the action of certain myelotoxic substances.

2. A high-fat, carbohydrate-free diet which, although adequate in protein and total calories, failed to support growth, induced profound but not irreversible changes in the content of nucleic acid and lipid phosphorus in bone marrow.

3. The following changes were found in the bone marrow of rats exposed to single X-ray doses ranging from 300 to 800 r. (a) The content of nucleic acid phosphorus: immediately after irradiation there were no obvious changes, except perhaps with the highest dose; considerable and progressive breakdown, proportional to the dose, was notice-

able a few hours after irradiation, reaching a maximum at 2 days; 7 days after exposure, up to 75 and 50 % recovery was recorded following 300 and 600 r., respectively, but it was much less advanced in the bone marrow of animals which survived 800 r.; at 10 days the pre-irradiation level was regained with the smallest dose, and further marked improvement was observed in those rats which had been irradiated with 500–600 r. (b) The content of lipid phosphorus was much less affected than nucleic acid phosphorus, with all doses and at all stages, except at 2 days with 800 r., when little lipid phosphorus remained. (c) Ascorbic acid, in a manner analogous to that of nucleic acid phosphorus, decreased in the early, and returned to normal value in the later, post-irradiation period. (d) A distinct diminution in the content of glutathione was demonstrated 2 days after irradiation with 500 r., but near-normal values were found at 7 days.

4. The general pattern of post-irradiation changes in the spleen conformed qualitatively to that of the bone marrow, but quantitatively it was much less pronounced.

5. Muscle adenylic acid and yeast adenylic acid, but not inosinic acid, appeared to delay the recovery of nucleic acid phosphorus and ascorbic acid content in the bone marrow and spleen of irradiated animals.

6. The phosphorus distribution of the bone marrow in splenectomized rats examined at various intervals after the removal of the spleen was indistinguishable from that established in unoperated controls; the same was true concerning the response of splenectomized rats towards X-rays.

7. Mild-degree burns of a limited area of skin did not exert any obvious effects on the phosphorus or ascorbic acid content of bone marrow or spleen.

8. Aminopterin and amethopterin selectively affected the bone marrow and had little or no effect upon the spleen. Similar findings were made with colchicine. On the other hand, mustard sulphoxide potentiated by dimethyldithiocarbamate acted, like X-radiation, upon the bone marrow as well as on spleen. Prolonged administration of amidopyrine had no significant effect on rat bone marrow or spleen.

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