sufficient to set up a continuous clinical syndrome which is indistinguishable from that occurring "naturally."

The experimental work of Rich (1945) is of special interest. He demonstrated changes in cardiac muscle and skin tissues seemingly identical to those of rheumatic fever when repeated injections of antigenic horse serum were given. However, it has not been possible to reproduce the whole clinical syndrome of a self-perpetuating rheumatic fever syndrome by these or any other means (Holbrook, 1953). We believe that under certain circumstances such a syndrome very occasionally follows inoculation procedures.

Whilst it is noted that the majority of delayed reactions of all types have followed a combined T.A.B.T. and vaccination procedure, similar disturbances have often been seen after either has been given separately. The reactions to vaccination are too well known to require description, but as these generally occur after about the same time interval—that is, seven to ten days—many of the cases have presented a rather confused pattern owing to the fever and malaise from the vaccination becoming superimposed upon the allergic reaction. In point of fact, it is quite possible that many of the delayed symptoms from vaccination are actually allergic.

A further clinical observation, though not statistically substantiated, is that infective conditions of all kinds, including upper respiratory infections, segmental pneumonias, and skin and tissue infections, are more common during the seven to ten days following inoculation, and this may help to make recognition of an underlying cause rather difficult. A possible explanation of this observation is that all the immunological processes are disturbed at this time, giving rise to a general lowering of resistance to infection.

We have, so far, mentioned only T.A.B.T. and vaccination reactions, but we do not believe that these are in any way due to specific antigens; it is merely that we have been able to observe so many delayed reactions because of the enormous scale of the procedures. We have seen occasional rheumatic syndromes after Mantoux-testing and also during the course of a delayed pulmonary reaction to lipiodol bronchography. It is interesting to note, in this connexion, that one of us has, in fact, suffered both a delayed pulmonary reaction to lipiodol (Roberston and Morle, 1951) and also a delayed allergic arthropathy to a Mantoux test.

Summary

Clinical observations have been made upon the reactions occurring one to two weeks after T.A.B.T. injection and vaccination and certain other procedures. These may involve the joints, closely resembling acute rheumatic fever or acute rheumatoid arthritis. There may actually be an apparent rheumatic carditis. A syndrome resembling periarteritis nodosa following inoculation is described. Various purpuric manifestations, identical with the so-called immuno-allergic vascular purpuras, are discussed. Changes in the reticulo-endothelial system, including spleen, liver, lymph nodes, and bone marrow, are described.

The significance of these delayed reactions is discussed, especially in connexion with the aetiology of the rheumatic and purpuric disorders.

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QUANTITATIVE EXAMINATION OF BONE MARROW IN GUINEA-PIGS AFTER GAMMA IRRADIATION

A PRELIMINARY NOTE

BY

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Previous work on the quantitative study of the bone marrow of the guinea-pig has shown that there are three main cell groups—myeloid, erythroid, and lymphocytic. The lymphocytes were found to constitute an unexpectedly high proportion of the total nucleated cells (Harris *et al.*, 1954). In a recent series of 10 normal animals (Harris and Harris, 1956), out of a total nucleated count of 1,814,000 ($\pm 86,000$) per c.mm. marrow there were about 430,000 ($\pm 42,000$) lymphocytes, or just under 25%, a figure which agrees closely with that of Sawitsky and Meyer (1948). So large a number of lymphocytes cannot possibly be due to contamination of the marrow with blood (Yoffey, 1954).

The question at once arises whether the presence of all these lymphocytes in the marrow is a purely incidental phenomenon, or whether they may function as stem cells for the myeloid and erythroid series. On this, perhaps the most controversial of all problems in haematology, it was felt that a quantitative analysis of marrow changes in conditions of severe marrow damage and subsequent regeneration should yield more precise information than has hitherto been available. If lymphocytes really can function as stem cells, then under these circumstances the course of the recovery should be associated with significant changes in the marrow lymphocytes.

Despite the very extensive investigations which have already been made upon the effect of irradiation on the haemopoietic system, some initial experiments indicated that a quantitative technique would make possible a much more precise analysis of the changes occurring. This communication presents the results of further investigations along these lines.

Material and Method

The dose of irradiation chosen was such that it produced marked changes in the haemopoietic organs, but at the same time involved as small a mortality rate as possible. For the guinea-pig, the minimum lethal dose for 50% of animals within 30 days of irradiation is said to be approximately 200–250 r (Ellinger, 1945). A dose of 150–170 r was ultimately selected as the dose that best fulfilled the above requirements. Marked changes in the bone marrow have occurred when using this dose, and in addition distinct cellular simplification has been achieved at a certain stage in recovery.

All animals used were males of a standard strain (Dunklin-Hartley) and about 400 g. in weight at the time of irradiation. A preliminary blood count was made, using blood obtained from an ear vein. Total R.B.C., W.B.C., and reticulocyte counts were made, together with a differential white cell count and haemoglobin estimation. An interval of about 10 days then elapsed, after which the animal was irradiated. Total-body irradiation was given, a cobalt-60 unit being the source of irradiation. It was performed under general anaesthesia (intraperitoneal pentobarbitone sodium) to ensure uniform dosage.

In each animal at a certain period after the time of irradiation a second blood investigation was made, followed immediately by quantitative examination of the bone marrow, using the technique described in previous papers from this department (Yoffey, 1955; Harris, 1956). Differential marrow cell counts were made upon smears fixed in methyl alcohol and stained by MacNeal's tetrachrome stain. One thousand cells were counted in each experiment.

Observations were made at daily intervals after irradiation until the fourth day and thereafter on alternate days up to the 28th day, with final observations on the 35th day.

Results

The results given are based upon 79 experiments, and further work is proceeding. The number of experiments in each group is shown in the accompanying Table. Comparison is made with the mean values obtained by quantitative examination of the bone marrow in ten normal animals whose mean weight was about 400 g.

Table Showing Number of Experiments and Time of Observation

Days: ..	1	2	3	4	6	8	10	12	14	16	18	20	22	24	26	28	35
No. ..	5	4	4	4	3	5	5	4	6	6	6	4	6	2	4	6	5

Total Nucleated Cells

Our experiments show that the count is already reduced below normal by the first day after irradiation. The lowest level is reached by the sixth day, being 674,000 per c.mm. marrow, which is approximately one-third of normal. This is followed by a gradual return to normal by the 20th day. However, though the number of cells is approximately back to normal, differential counts reveal that there is a far from normal distribution of the main cell groups at, and for some days after, this stage.

Myeloid Cells

The vast majority of cells in this group are neutrophils. Twenty-four hours after irradiation there is initially a slight increase in the total myeloid cells which is due to the presence of increased numbers of mature or nearly mature cells (562,000 band and mature neutrophils per c.mm. marrow, compared with 431,000 per c.mm. in the controls). The more immature cells are decreased at this time. After this the number of cells decreases, reaching a very low figure by the 14th day, being 47,000 per c.mm. marrow. By the 18th day granulocytes are beginning to reappear in the marrow in large numbers, and have reached almost normal levels by the 20th day after irradiation. The increase continues, and reaches well above normal by the 22nd and 24th days (1,130,000 and 1,146,000 per c.mm. respectively). Thereafter there is a decrease to more normal values.

Subdivision of the neutrophils into two groups—immature (that is, metamyelocyte or earlier forms) and mature (that is, band and segmented cells)—shows that the great majority of cells present up to the 14th day are of the mature variety. In the interval between the 12th and the 14th day after irradiation there is a rather sharp fall, reaching as low as 6,000 cells per c.mm. It is of interest that a marked neutropenia occurs in the blood from the 14th to the 18th day after irradiation.

A rapid re-population of the marrow with immature neutrophils begins on the 16th day, and within a period of two days they have risen to well above normal. A peak level is reached on the 20th day after irradiation, when the number of immature cells is approximately double that of the normal. After this time the level of immature cells gradually returns towards normal.

The more mature forms of neutrophils begin to reappear in the marrow between 18 and 20 days after irradiation, reaching their maximum between the 22nd and 24th days.

At this time their level in the marrow is about twice normal. Subsequently the mature neutrophils return to more normal figures over a period of four days.

Erythroid Cells

The erythroid precursors would appear to be very sensitive to irradiation, and even by 24 hours there is a low content of these cells, which at this time are less than one-third of normal. Low levels (less than 100,000 per c.mm.) persist until the 12th day, after which they rise to normal within a period of four days. A continued elevation of these cells occurs, reaching a maximum (about twice normal) by the 20th day. There then follows a decline to normal levels.

Lymphocytes

These cells, like the erythroblasts, are very sensitive to irradiation, and after 24 hours their number in the marrow is less than one-third of the normal value. A low level persists until the 4th day, but after this time the marrow lymphocytes tend to rise gradually, and have exceeded the normal figure by the 10th day. They continue to increase until by the 16th day their numbers have approximately doubled in comparison with the controls. After the 16th day the lymphocytes fall quite rapidly to subnormal values, minimal levels being reached on the 22nd and 24th days after irradiation (approximately one-third of normal). However, by the 28th day the number of lymphocytes in the bone marrow has again returned to normal.

Discussion

There have been two outstanding findings in these experiments. Firstly, the lymphocyte, although very sensitive to irradiation, recovers quickly relative to the erythroid and myeloid series. A level well above normal is reached between the 14th and the 16th day after irradiation, and this

FIG. 1.—Erythroid, myeloid, and lymphocyte changes in guinea-pig marrow after gamma irradiation.

marked accumulation of lymphocytes precedes the regeneration of cells in both the erythroid and the myeloid groups (Fig. 1). Furthermore, at this time of high lymphocyte content in the marrow numerous lymphocyte-like cells appear with a characteristic morphology. These we describe as transitional cells (Yoffey and Courtice, 1956). The observations appear to confirm those of Brecher *et al.* (1948),

who investigated the effects of x rays on the lymphoid and haemopoietic tissues of albino mice. These authors noted that lymphocytopoiesis appeared to be normal by the 7th and 10th day after irradiation, and even to be raised above normal by the 14th day. An abrupt temporary rise in marrow lymphocytes occurred on the 10th day and accompanied a persistent blood lymphopenia. A similar relationship between blood and marrow lymphocytes has been noted in these experiments. Brecher *et al.* also describe the appearance of a distinct cell type, a lymphocytic-like cell (resembling the lymphoidocyte of Pappenheim) which they suggest is a stem cell of the granulocytic series.

The second finding is that the great decline in marrow lymphocytes occurring after the 18th day corresponds closely with the presence of large numbers of both erythroid and myeloid cells in the marrow (Fig. 2). An inverse relationship between the levels of mature neutrophils and lymphocytes in the bone marrow has been noted in a quantitative study of the effect of leucocytosis-promoting factor on guinea-pig bone marrow (Harris *et al.*, 1956).

A remarkable increase in the immature forms of both myeloid and erythroid cells occurs over the short period of two to four days. Since there is no obvious increase in the number of mitotic figures, and in view of the marked decline (following a peak) in lymphocyte content of the marrow, it is reasonable to postulate a direct relationship between the accumulation of lymphocytes and the recovery of the other cell series. The appearance of forms of cell having a transitional appearance between a typical small lymphocyte and the blast precursors of myeloid and erythroid cells further supports this hypothesis.

The low level of erythroid precursors in the bone marrow 24 hours after irradiation is identical with that of the lymphocytes. This is in agreement with the findings of Bloom and Bloom (1947), who investigated the effects of

FIG. 2.—Changes in guinea-pig marrow after gamma irradiation: showing decline in marrow lymphocytes after the 18th day, corresponding closely with the presence of large numbers of myeloid cells.

irradiation on haemopoietic tissues in various animals. They conclude that the erythroblast is as sensitive to irradiation as the small lymphocyte. Recovery of the erythroid series in the present experiments precedes that of the myeloid series by an interval of between two and four days. Rosenthal *et al.* (1951) in a semi-quantitative study of bone marrow in rats following total-body x-irradiation, noted that there was evidence of erythroid regeneration 12 days after irradiation, compared with 15 days for the myeloid series. They also remarked that from the 12th day onwards the bone-marrow lymphocyte count increased more than the blood level. Valentine and Pearce (1952) investigated the relative sensitivity of erythroid and myeloid elements in cats after total-body irradiation. They concluded that erythroid tissue is less sensitive to irradiation than myelopoietic tissue, as gauged by recovery times.

The initial rise of mature and nearly mature neutrophils in the marrow occurring 24 hours after irradiation has been noted by other workers (Aubertin and Beaujard, 1908;

Rosenthal *et al.*, 1951; Latta and Waggener, 1954). Rosenthal *et al.* remarked that the fall in myeloid elements of the rat bone marrow occurring 24 hours after irradiation involves the more immature cells, there being a slight rise in the more mature, segmented, cells at this stage. The sharp fall in the level of mature neutrophils occurring in the interval between 12 and 14 days after irradiation suggests that the duration of life for immature neutrophils within

FIG. 3.—Changes in mature and immature neutrophils in guinea-pig marrows after gamma irradiation.

guinea-pig bone marrow is at least 14 days. Restoration of granulocytes occurs with such remarkable rapidity that within four days they have increased from 47,000 per c.m.m. to 482,000 per c.m.m. Comparison between the peaks of immature and mature neutrophils in the marrow (Fig. 3) shows that they are separated by an interval of between two and four days. It therefore appears that the time taken under these conditions for immature neutrophils to mature in the bone marrow is from two to four days.

Summary

Quantitative examinations of bone marrow in the 400-g. male guinea-pig have been made at varying intervals up to five weeks after total-body irradiation.

The results confirm the well-known fact that the lymphocyte is very sensitive to radiation, but also indicate that there is a marked accumulation of these cells in the bone marrow between 10 and 18 days after irradiation. This accumulation occurs at the same time as a lymphocytopenia in the blood. The presence of a large number of lymphocytes in the marrow precedes the recovery of the stem cells in both the myeloid and the erythroid series. Accompanying the accumulation of lymphocytes, numerous cells are seen which morphologically appear to be transitional between the lymphocyte and the typical blast precursors of the myeloid and erythroid series. The significance of the findings is discussed.

During the marrow regeneration, erythropoiesis seems to reach a peak at about the 20th day, and myelopoiesis between the 22nd and 24th days. At the period of sustained erythropoiesis and myelopoiesis the inverse relation to the marrow lymphocytes, which fall precipitously, is very striking.

Regeneration of cells in the myeloid group begins two to four days after that of the erythroid group. Evidence suggests that the time taken for immature neutrophils to mature in guinea-pig bone marrow in the recovery from irradiation is in the region of two to four days.

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BLOOD PYRUVATE AND α -KETOGLUTARATE IN NORMAL AND DIABETIC SUBJECTS

BY

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The blood concentrations of α -keto acids in patients with diabetes mellitus have been the subject of conflicting reports. Thus Gey (1953) observed raised blood α -ketoglutarate levels in some diabetic patients, and Villano (1952) and Seligson and Shapiro (1952) stated that the blood concentrations of both pyruvate and α -ketoglutarate were increased in diabetes. Other workers (Bueding *et al.*, 1942b; Klein, 1942) reported that the fasting blood pyruvate was normal in diabetic patients, and Lestradet and Guest (1951) and van Marken Lichtenbelt and Florijn (1952, 1955) found normal blood pyruvate values in diabetic acidosis and coma. In a preliminary communication (Smith and Taylor, 1955) we reported that the blood concentrations of pyruvate and α -ketoglutarate, measured by a specific paper chromatographic method, were normal in a group of ambulant diabetic patients. This work has now been extended to a larger number of diabetic patients, including those with more severe grades of the disease.

The fluctuations in the blood pyruvate after the administration of glucose to diabetic patients have been studied by several workers. Most of them (Himwich and Himwich, 1946; Klein, 1942; Bueding *et al.*, 1942a; Miller *et al.*, 1952) reported that diabetics showed little change in blood pyruvate after glucose, but the results of Root *et al.* (1946) and Amatuzio *et al.* (1952) suggested that diabetic patients did not respond as a uniform group in this test. In general, patients with a mild form of the disease showed a rise in blood pyruvate, whereas in patients with severe diabetes this rise was either delayed or absent. It is now generally accepted that diabetes mellitus is not a single disease entity, and Lawrence (1951) has distinguished two main clinical

types—the obese patients not requiring insulin and the thin insulin-requiring patients. It therefore seemed of interest to investigate the effect of the administration of glucose on the blood concentrations of pyruvate and α -ketoglutarate in these two clinical types of diabetic patients.

Experimental

Analytical Methods.—The blood concentrations of pyruvic and α -ketoglutaric acids were measured by the method of Taylor and Smith (1955), except that tungstic acid, in the amounts used by Seligson and Shapiro (1952), was substituted for metaphosphoric acid as the protein precipitant. This modification reduced emulsion formation during the extraction procedure and slightly improved the recoveries of the α -keto acids. All the blood specimens were obtained from arm veins, no clenching or unclenching of the fist being allowed before or during collection. Blood glucose was estimated by the method of Nelson (1944) on the same venous blood samples.

Clinical Material.—Single blood samples were obtained from 12 normal subjects and 17 ambulant diabetic patients attending the out-patient clinic. The blood specimens were collected approximately three hours after breakfast, and a sample of urine was also obtained from each diabetic patient at the same time and tested for ketone bodies. The diabetic patients were taken at random and were free from disease apart from their diabetes.

Oral Glucose Test.—Twelve normal subjects and 16 diabetic patients were fasted overnight and urine samples were obtained from the diabetic patients and tested for ketone bodies. Each subject was allowed to rest on a bed for one hour before the first (0 hour) specimen of blood was collected. A 25% (w/v) solution of glucose (equivalent to 1.75 g. of glucose per kg. body weight) was then given by mouth and blood specimens were collected at intervals of one, two, and three hours. Thirteen of the diabetic patients were divided into two main groups according to Lawrence (1951), and the details of the patients are given in Table I. The first group ("obese" group) comprised six patients who gave a history of recent gain in weight or who had maintained weight on a low-calorie diet and were not ketosed.

TABLE I.—Classification and Details of the Diabetic Patients Used for Oral Glucose Test

Case No.	Sex	Age	Recent Weight Change	Treatment	Urine Test	
					Rothera	Gerhardt
<i>Obese Group</i>						
1	F	23	Gain	New patient	0	0
2	M	59	Maintained	Diet	0	0
3	F	53	"	New patient	0	0
4	F	46	Gain	" "	0	0
5	F	52	Maintained	" "	0	0
6	F	62	"	Diet	0	0
<i>Thin Group</i>						
7	M	26	Loss	Insulin	0	0
8	M	33	"	"	0	0
9	M	34	"	New patient	+++	0
10	F	55	"	Insulin	++	0
11	M	30	"	New patient	+++	++
12	M	46	"	" "	+++	++
13	M	33	"	" "	+++	+

The second group of seven patients ("thin" group) gave a history of great loss of weight and severe ketosis before insulin therapy; four patients in this group were new untreated patients and the remainder were under treatment, their insulin being stopped for 24 hours before the test. A further small group of three obese diabetic patients showing a recent significant loss of weight was also studied.

Results

The blood pyruvate, α -ketoglutarate, glucose, and urine ketone results for the individual diabetic patients, together with the mean results for the normal subjects, are given in Table II. In all the following tables the significances of