HEMOPOIETIC COLONY STUDIES

V. Effect of Hemopoietic Organ Stroma on Differentiation of Pluripotent Stem Cells*

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Plates 30–36

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When appropriate doses of viable bone marrow cells (10^4–10^5) are injected into lethally irradiated mice, distinct nodules appear in the spleen of the recipient, representing foci of donor cell hemopoietic proliferation (1). Evidence based upon marker chromosome distribution indicates that each such nodule of developing hemopoietic cells (spleen colony) is clonal in origin, i.e., it is the progeny of one cell designated as a "colony forming unit" (CFU) (2). The CFU may or may not always represent a true stem cell. In their earlier stages of development (up to 7 or 8 days), most spleen colonies are of either erythropoietic or granulopoietic nature, not both. Soon after this period, however, nearly all colonies contain cells of both the erythropoietic and granulopoietic series (3, 4). Transfusion of cells from an erythropoietic colony into a newly irradiated recipient gives rise to both erythropoietic and granulopoietic spleen colonies. The ratio of the two colony types in the secondary recipient is nearly identical to the ratio in the primary host (4, 5).

Suppression of erythropoietin secretion by hypertransfusion-induced polycythemia results in suppression of development of the erythroid spleen colonies, but not of the granuloid colonies. The suppressed presumptive erythroid colonies remain small and undifferentiated *but do not become granuloid colonies.* To resolve these apparently opposing observations, we have theorized that the spleen is subdivided into a variety of microenvironmental areas, each inducing a single type of differentiation on the part of otherwise pluripotent stem cells (5). The present investigation represents an attempt to prove or disprove this working hypothesis by an extension of our hemopoietic colony studies from the spleen to the bone marrow.

In the spleens of lethally irradiated, bone marrow-injected mice, the ratio of erythroid to granuloid colonies (E:G ratio) is approximately 3 to 1 (4, 5, 6, and Table II). In the bone marrow, granulopoiesis is known to exceed erythropoiesis. What would be the E:G colony ratio of hemopoietic colonies forming in the bone marrow? If it differs from the E:G colony ratio of the spleen,

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is the difference caused by selective migration of committed CFU's to these two organs, or by an influence of the organ stroma on the differentiation of pluripotent CFU's?

Materials and Methods

(C57 × A)F_1 (males and females in Tables I and II, males in Table III, females in Table IV) received X-irradiation from a 250 kvp or a 300 kvp therapy machine operated at 200 kvp (15–30 ma, 1 mm Al + 0.5 mm Cu filters, half-value layer 1.05 mm Cu for Table I; 1.2 mm Cu for Tables II, III, and IV). Irradiated mice which were to survive 7 days or longer were supported by daily subcutaneous injections of 0.8 mg gentamicin (Garamycin sulfate, Schering Corp., Bloomfield, N.J.) plus 4 mg streptomycin in saline. Cell suspensions were made in Gey's solution containing penicillin G, 1000 USP units per milliliter and streptomycin sulfate, 1 mg/ml. The dose of viable isogenic cells injected was determined by (nigrosin) dye-exclusion hemocytometer counts. Bone marrow was given by the intravenous route except where otherwise noted. The method of trocar-transplantation for the recipients listed in Tables III and IV will be described below. The spleens plus five bones (two femurs, two tibias, and the sternum) from each recipient mouse were fixed in 10% formalin. The bones were decalcified, mounted in a single paraffin block, and subserially sectioned. Five to nine equally spaced longitudinal sections were examined microscopically. Spleens were subserially sectioned and 5 or 10 equally spaced longitudinal sections examined. Hematoxylin and eosin staining was used. Criteria of colony identification and quantitation were as previously described (3, 5).

TABLE I

Endogenous and Exogenous Colonies in Bone Marrow and Spleen of 1000 R X-Irradiated Mice

Procedure	No. mice	Day of sacrifice	Marrow colonies*							Spleen colonies	
			E	G	M	U	Meg	No. colonies per mouse	E:G	No. colonies per mouse	E:G
								average		*average*	
1000 R only	21	7	1	21	0	0	1	1.1	0.05	0.0	—
1000 R only	10	10	0	2	0	1	0	0.4*	0.0	0.0	—
1180 R only	11	8	0	3	0	1	0	0.4	0.0	0.0	—
1000 R + 4 × 10^4 viable BM cells‡	6	7	6	13	1	1	0	3.5	0.5	13.7§	2.3
1000 R + 1 × 10^5 viable BM cells	12	7	31	57	15	6	0	9.1	0.5	16.2	5.0

* Five bones (two femurs, two tibias, and the sternum) were examined by subserial section for each mouse, except the 10 mice of the second row above. A correction factor of 5/4 was applied in the average colonies/mouse column for this group since only the 4 long bones were examined. E, erythroid; G, granuloid; M, mixed; U, undifferentiated; Meg, megakaryocytic; E:G, number of erythroid colonies ÷ by number of granuloid.

‡ BM, bone marrow.

§ This is an unusually high yield of spleen colonies for the amount of marrow injected. No procedural factor could be found to explain it.

RESULTS

Control mice which received 1000 R had approximately one endogenous colony per five bones examined. In mice receiving 1180 R, endogenous colonies approximated 0.4 per five bones (Table I). No colonies were found in the spleens

TABLE II

Colony Types in Bone Marrow and Spleen in 1190 R Irradiated Mice Injected I.V. with 6 × 10^4 Viable Bone Marrow Cells*

Experimental group	No. mice in group	Tissue examined	No. colony types			E:G ratio
			Erythroid	Granuloid	Mixed	
			average	*average*	*average*	
I. Mice without S.C.* whole spleen transplants	26	Own spleen	5.7	2.0	1.0	2.8
		Bone marrow	1.0	1.8	0.3	0.6
II. Mice bearing 4 S.C. whole spleen transplants each	11‡	Own spleen	4.8	0.7	1.2	6.6
		S.C. spleens	4.3	1.2	0.1	3.6
		Bone marrow	1.1	2.3	0.6	0.5
III. Mice bearing 8 S.C. whole spleen transplants each	7	Own spleen	6.0	1.9	0.7	3.2
	7	S.C. spleens	5.3	1.0	0.1	5.3
		Bone marrow	2.3	1.4	0.3	1.6
IV. Mice bearing 16 S.C. whole spleen transplants each	7	Own spleen	6.4	1.3	0.6	5.0
		S.C. spleens	12.7	4.3	0.6	2.9
		Bone marrow	0.7	1.1	0.3	0.6
Sums of above groups	51§	Own spleen	5.6	1.6	1.0	3.5
		S.C. spleens	6.9	2.0	0.2	3.5
		Bone marrow	1.2	1.7	0.3	0.7

* I.V., intravenously; S.C., subcutaneous.

‡ Due to a histology lab accident, only 8 of these mice furnished bones for examination.

§ In summation, 51 own (in situ) spleens, 104 S.C. transplant spleens, and 235 bones were studied by semiserial section.

Frequencies of erythroid versus granuloid colonies were compared by the χ^2 method. $P = <0.001$ when either in situ spleen or S.C. spleens are compared against bone marrow, using either sums of all experimental groups or group I only.

at either dose of irradiation. The endogenous bone colonies were almost invariably granulocytic in nature (Fig. 1) and most were smaller than granulocytic colonies arising from exogenous CFU's.

Discrete foci of hemopoietic regeneration were found in the sections of bone marrow of irradiated mice receiving low doses of bone marrow cells intravenously (Figs. 2, 3). Their numbers were directly proportional to the

number of marrow cells injected. At 7 or 8 days, most of them were composed of a single line of hemopoietic differentiation, usually granulocytic. Their time of appearance was comparable to that of spleen colonies, like which they are presumed to be of clonal origin. They were smaller than 7 or 8 day spleen colonies. This was especially true for the erythroid colonies which, in bone marrow, were found to be no larger than the granuloid colonies. In the spleen, the erythroid colonies are normally much larger than the granuloid colonies (3). The limitation of size was not due to limitation of marrow space at this time, since the individual colonies often did not extend the full width of the marrow cavity. The cell types found were at least as mature as those in well developed spleen colonies. Colonies with predominantly immature cells were a rarity. In con-

TABLE III

7-Day Colony Types Developing from Reseeded CFU's Present in Marrow Stroma Inserted by Trocar into Spleens of 1000 R Irradiated Secondary Recipients

No. mice in group	CFU route of entry	Colony contact with trocar-implanted marrow stroma	Colony types in spleens*						Colony types in five bones taken from same mice					
			E	G	M	U	Meg	E:G‡	E	G	M	U	Meg	E:G
		%												
28	Reseeded marrow stroma trocar-implanted into spleen	>50	4	38	6	2	0	0.1	7	26	1	1	2	0.3
		<50	41	14	17	2	1	2.9						
		no contact	31	13	0	7	3	2.4						
30	Contralateral BM suspension I.V.	—	247	87	25	20	12	2.8	42	68	26	1	3	0.6

* E, erythrocytic; G, granulocytic; M, mixed; U, undifferentiated; Meg, megakaryocytic.

‡ E:G, ratio of erythroid colonies to granulocytic colonies.

The frequencies of E colonies versus G colonies, and of E colonies versus all other types of colonies in the spleens, were compared between the >50% contact group and each of the other groups (<50% contact, no contact, and BM suspension I.V.). Using the χ^2 test, P = <0.001 that there is no difference in these frequencies.

trast to spleen colonies, the average E:G colony ratio in the marrow cavity of marrow-injected animals was less than 1, usually 0.5 to 0.7 (Tables I and II).

The experiments described in Table II were originally designed to show distribution and percentage recovery of injected CFU's in the various hemopoietic organs. Mice of several of the groups were subcutaneously transplanted with 4, 8, or 16 isogenic adult whole spleens 60 days previous to irradiation and bone marrow cell injection. Included, however, is a composite control group, bearing no spleen transplants and injected with the same bone marrow preparations. This group shows essentially the same E:G colony ratio in the bone marrow as do the spleen-transplant bearing groups. The average E:G colony ratio was 0.7 for colonies in the marrow cavity, and 3.5 for those in the spleens, either in situ or subcutaneously implanted. Mixed colonies represented 10%

of all colonies in the bone marrows, 11.6% of those in the spleens in situ, and 2.6% of those in the subcutaneous spleens. Mixed colonies were excluded in calculating the E:G colony ratio. There was, in addition, a small scattering of megakaryocytic colonies and of colonies too undifferentiated to classify, which taken together represented 4–7% of all colonies seen. The difference between the E:G colony ratio in the marrow cavity and in the spleens was 5-fold and statistically was highly significant. The transplanted spleens (which before irradiation were themselves each only about one-fifth the size and weight of a normal spleen) contained colonies which were also smaller than those in the spleens in situ. The colonies in the marrow cavity were similar in size to the exogenous colonies of Table I.

Table III presents the pooled data of three separate experiments in which the results were comparable. The 1000 R irradiated primary recipients were given a single intravenous injection of from 2.5×10^6 to 3×10^7 viable bone marrow cells. These mice were sacrificed 18–24 hr postinjection and the content of their femoral marrow cavities then used to repopulate 1000 R irradiated secondary recipients in the following manner: From each primary recipient individually, as much as possible of a single femoral marrow stroma (usually ¼-½, obtained by cracking open the bone) was implanted by 18 gauge trocar directly into the spleen of a secondary irradiated recipient. The marrow cells from the opposite femur of the same primary recipient were suspended by flushing in 1 cc of Gey's solution, and injected intravenously into another secondary irradiated recipient. In one of the three pooled experiments which constitute Table III the control mice injected intravenously with marrow cells were subjected to a sham operation, with insertion of an empty trocar into the spleen. Since no meaningful differences were observed, the results of the three experiments were pooled.

The boundary of the trocar-implanted marrow stroma in the spleens of the secondary recipients was sharply demarcated, microscopically, by textural differences in connective tissue and by the presence of numerous bone spicules of the marrow stroma (Fig. 4). The marrow stromal implants were not encapsulated and appeared in intimate contact with the adjacent splenic tissue. Three classifications were set up for the relationship of colonies to the implanted marrow stroma. The first classification (Table III) contains those colonies in which 50% or more of the colony was within the marrow stroma, and was presumed to have developed from a CFU remaining lodged therein. The second contains those colonies in which less than 50% of the colony was in contact with the marrow stroma and which very likely developed from a CFU that had migrated and lodged just outside the marrow stromal area. The third contains those colonies not at all in contact with the marrow stroma and which, necessarily, developed from CFU's which wandered some distance from it during or after the implant by trocar. Not all of the col-

onies placed in the >50% contact or <50% contact groups of Tables III and IV were seen to be occupying both sides of the border of the marrow stroma insert. Some of the former were 100% within the confines of the marrow stroma and many of the latter were only in contact with the borderline, but could not be seen to extend into the marrow stroma.

The control secondary recipients, each of which were injected intravenously

TABLE IV

*7-Day Colony Types from Marrow or Splenic CFU's Developing in Either Spleen or in an Intrasplenic Implant of Marrow Stroma**

No. mice in group	Treatment	Colony contact with trocar-implanted marrow stroma	Colony types in spleens‡					
			E§	G	M	U	Meg	E:G
		%						
4	2×10^5 unirradiated bone marrow cells I.V. (control)	(No marrow trocar implant)	41	15	10	0	2	2.7
5	2×10^5 unirradiated bone marrow cells I.V. plus 1000 R irradiated piece of bone marrow trocar-implanted into spleen	>50	1	1	0	0	0	1.0
		<50	21	3	1	0	0	7.0
		no contact	45	10	9	0	6	4.5
6	1 mm piece of unirradiated spleen and 1000 R irradiated piece of bone marrow trocar-implanted into spleen	>50	0	5	2	0	0	0.0
		<50	15	0	9	0	1	>15.0
		no contact	40	14	3	0	3	2.9
11	1000 R irradiated piece of bone marrow implanted into spleen (control)	>50	0	0	0	0	0	—
		<50	0	0	0	0	0	—
		no contact	0	0	0	0	0	—

* All donors of marrow stroma trocar-implants and all recipient mice received 1000 R whole body irradiation.

‡ Colony numbers are probably an underestimate due to semiconfluency of colonies in these spleens.

§ E, erythrocytic; G, granulocytic; M, mixed; U, undifferentiated; Meg, megakaryocytic; E:G, ratio of erythroid colonies to granulocytic colonies.

with donor marrow cells flushed from one of the primary recipient's femurs and suspended in Gey's solution, had the usual E:G colony ratio (2.8) in their spleens (Table III). In those secondary recipients which received the marrow stroma of the other femur implanted directly into their spleens via trocar, the colonies which developed predominantly in contact with the marrow stroma were nearly all only granulocytic in type (E:G colony ratio = 0.1). However, most of those colonies which developed initially or entirely in contact with the

surrounding splenic stroma were erythroid (E:G colony ratio = 2.9 and 2.4). The E:G colony ratios in the bones of both the marrow stroma–implanted and the intravenously injected mice were 0.3 and 0.6, respectively. The stroma-implanted mice had so few colonies in their bones that the majority of such colonies may have been of endogenous origin, resulting in a lower E:G colony ratio. A very low E:G colony ratio is characteristic of endogenous bone marrow colonies examined 7–10 days postirradiation (Table I).

Another procedure was attempted, in which CFU's from either bone marrow or spleen-derived cells were allowed to migrate into, rather than out of, the implanted marrow stroma. This was accomplished at first by inserting bone marrow stroma from 1000 R irradiated, noninjected mice into spleens of 1000 R irradiated recipients, 2–3 hr after intravenous injection of viable bone marrow cells into those same recipients. A second and more successful method was the loading of a 1 mm cube of normal (unirradiated) splenic tissue into the trocar together with the irradiated marrow stroma before insertion into the irradiated recipient's spleen. The results are shown in Table IV. Because of the small numbers of colonies involved and the finding of no erythroid colonies in one group with more than 50% contact with bone marrow stroma, and of no granuloid colonies in a group with less than 50% contact, the E:G ratios are extreme, but in the direction consistent with the working hypothesis. There was considerable confluency of those colonies in the area of the inserted bit of normal spleen, contributing to the higher incidence of mixed colonies, and making for less accuracy in colony counting. This was not sufficient to put in doubt the approximate E:G colony ratios, however. A control experiment showed that when the 1000 R irradiated stroma was implanted alone (without an accompanying source of unirradiated CFU's) no colonies developed in either the implant or the surrounding spleen (bottom line, Table IV).

The E:G colony ratios presented are calculated from colonies that are either erythroid or granuloid, not mixed. However, that group of mixed colonies which happened to straddle the junction of bone marrow implant and adjacent spleen were unexpectedly revealing. Not every mixed colony straddled the borderline. Not every one of the 30 colonies of the experiments of Tables III and IV that straddled the borderline was mixed. But of those 19 colonies that straddled the border line and were mixed, in every case that portion of the colony proliferating on the spleen side was predominantly erythroid, that portion on the marrow side was predominantly granuloid (Figs. 5, 6, and 7). There was often some mixture of erythroid and of granuloid cells at the area of changeover. Less frequently the transition of cell types was rather abrupt (Figs. 6 and 7).

DISCUSSION

These observations on hemopoietic colonies in regenerating bone marrow are consistent with, and therefore support, the working hypothesis derived

from earlier spleen colony studies regarding the influence of the organ microenvironment on the line of hemopoietic differentiation within a colony (5, 7). In the bone marrow cavity, in which granulopoiesis is known to exceed erythropoiesis, most early colonies were found to be granuloid, whereas in the spleen (where erythropoiesis is known to exceed granulopoiesis) most early colonies were erythroid. In irradiated mice bearing hemopoietic transplants, the E:G colony ratio in spleen is about 3 or more whether the spleen is in situ or transplanted subcutaneously (Table II). The E:G colony ratio in the bone marrow stroma is less than 1 (about 0.5–0.7) whether the marrow stroma is in situ or trocar transplanted into the spleen (Tables I, II, III, IV). That this is more likely due to an effect of the microenvironment on pluripotent stem cells of the developing colony, rather than to either a selective localization of committed erythroid or granuloid CFU's, or to local selective repression of one or the other type of committed CFU once lodged, is indicated by (*a*) much evidence that most (but probably not all) CFU's are pluripotent (5); (*b*) histological evidence that colonies alter from pure type to mixed as they enlarge and encounter new areas of splenic stroma (3); (*c*) the fact that CFU's derived from either normal bone marrow (Tables I, II, III, IV) or normal spleen (Table IV) or individually dissected erythroid or granuloid spleen colonies (5) give, on transplantation, all types of colonies, with an E:G colony ratio determined by whether the colonies develop in spleen or bone marrow; (*d*) when marrow stroma was implanted into the spleen the mixed colonies straddling the junction of marrow stroma and spleen were always predominantly (and often completely) granuloid on the marrow side and erythroid on the spleen side. This last finding confirms earlier transplantation studies indicating that developing colonies contain pluripotent CFU, not all of which are committed to the first microenvironment of residence (5, 7). It also supports our earlier observations and impressions that, when mixed differentiation of a colony does occur, the second line of differentiation usually occurs at the periphery and among the least differentiated peripheral cells of the largest colonies, presumably because of expansion into an adjacent microenvironment of a different type than the first. The finding that all of the mixed colonies occupying both sides of the demarcation between splenic stroma and inserted marrow were erythroid on the splenic side and granulocytic on the marrow side is not in accordance with a unipotent lineage of CFU's, for unipotent cells can, by definition, beget only unipotent cells. The question of local repression of unipotent CFU's is best answered by the dissected colony transplantations (4, 5), the results of which do not lend themselves to such an interpretation.

The findings reported here are most reasonably interpreted as further and direct evidence of a determining effect of the hemopoietic organ stroma upon a pluripotent CFU. We suggest the possibility that this pluripotent CFU may be the true hemopoietic stem cell and the immediate ancestor of a unipotent CFU. The relative proportions of these two CFU's presumably depend upon

local organ dynamics and upon the demands made upon the blood-forming system, but, under physiologic conditions, the multipotent CFU would appear to predominate in the bone marrow of the mouse and to be present in some numbers in the spleen. That "committed" CFU's do exist is indicated by the slightly higher percentage of erythroid colony progeny of transplanted erythroid colonies than of transplanted granuloid colonies (5).

Previous studies indicated that the hemopoietic organ stromal effect is of a microenvironmental nature, and that the spleen contains at least 4 types of microenvironments, determining differentiation of pluripotent cells into either erythroid, neutrophilic granuloid, megakaryocytic, or eosinophilic granuloid colonies, each occurring in distinctive pattern and frequency with the spleen (3, 5). The nature of the determining effect is presumed to be of a classical inducing nature, requiring close proximity or contact of inducing and induced cells. The term hemopoietic-inductive microenvironment (HIM) has therefore been applied (5). The mechanism of action of the erythroid HIM could be to induce a state of erythropoietin responsiveness.

SUMMARY

In heavily irradiated mice, bone marrow regeneration of either endogenous or exogenous origin was shown to occur in discrete foci comparable to the more intensively studied spleen colonies. The number of endogenous bone marrow colonies was inversely related to dose of whole body X-irradiation. Endogenous marrow colonies were found after higher doses of irradiation than were endogenous spleen colonies. Most of them were granulocytic in nature.

Exogenous bone marrow colonies in lethally irradiated mice injected with bone marrow cells were proportional in number to the dose of cells injected, appeared at a time comparable to spleen colonies like which, at 7 or 8 days, they were of single differentiated cell line, either granuloid or erythroid or megakaryocytic, with a small percentage of "mixed" colonies.

Whereas erythroid colonies outnumber granuloid colonies in spleen, either in situ or subcutaneously transplanted (E:G colony ratio of about 3.5), granuloid colonies outnumber erythroid in bone marrow (E:G colony ratio of about 0.7). The characteristic E:G colony ratios of spleen and marrow appear more likely to be the result of a hemopoietic organ stromal influence on pluripotent colony forming units (CFU's) than of selective lodgment of committed (unipotent) granuloid and erythroid CFU's in bone marrow and spleen, respectively, as indicated by the following.

Bone marrow stem cells (CFU) which had reseeded the marrow cavity of irradiated primary recipients 18–24 hr earlier, were reharvested and retransplanted intravenously into irradiated secondary hosts. The E:G colony ratio of the colonies formed in the spleen of the secondary hosts was typical of primary spleen colonies (2.8), that of the colonies formed in the marrow cavity was typical of bone marrow colonies (0.6). Pieces of marrow stroma containing re-

seeded CFU's from the contralateral femur of these same primary recipients were implanted by trocar directly into the spleens of other irradiated secondary recipients. Those CFU's that developed in the intrasplenic-implanted marrow stroma yielded an E:G colony ratio of 0.1. Those that migrated into the contiguous and remote portions of the spleen gave E:G colony ratios of 2.9 and 2.4, respectively.

Irradiated marrow stroma and normal spleen CFU's (a 1 mm cube of spleen) were loaded into the same trocar and implanted directly into the spleens of irradiated mice. The spleen CFU's that migrated into the implanted marrow stroma yielded five granuloid and two mixed colonies. The larger number that developed in the host spleen yielded an E:G colony ratio of 2.9 or higher.

Of those 19 mixed colonies that bridged the junction of spleen and implanted marrow stroma in each of the above two experiments, in every case, the erythroid portion of the colony was in the splenic stroma, the granuloid portion was in the marrow stroma.

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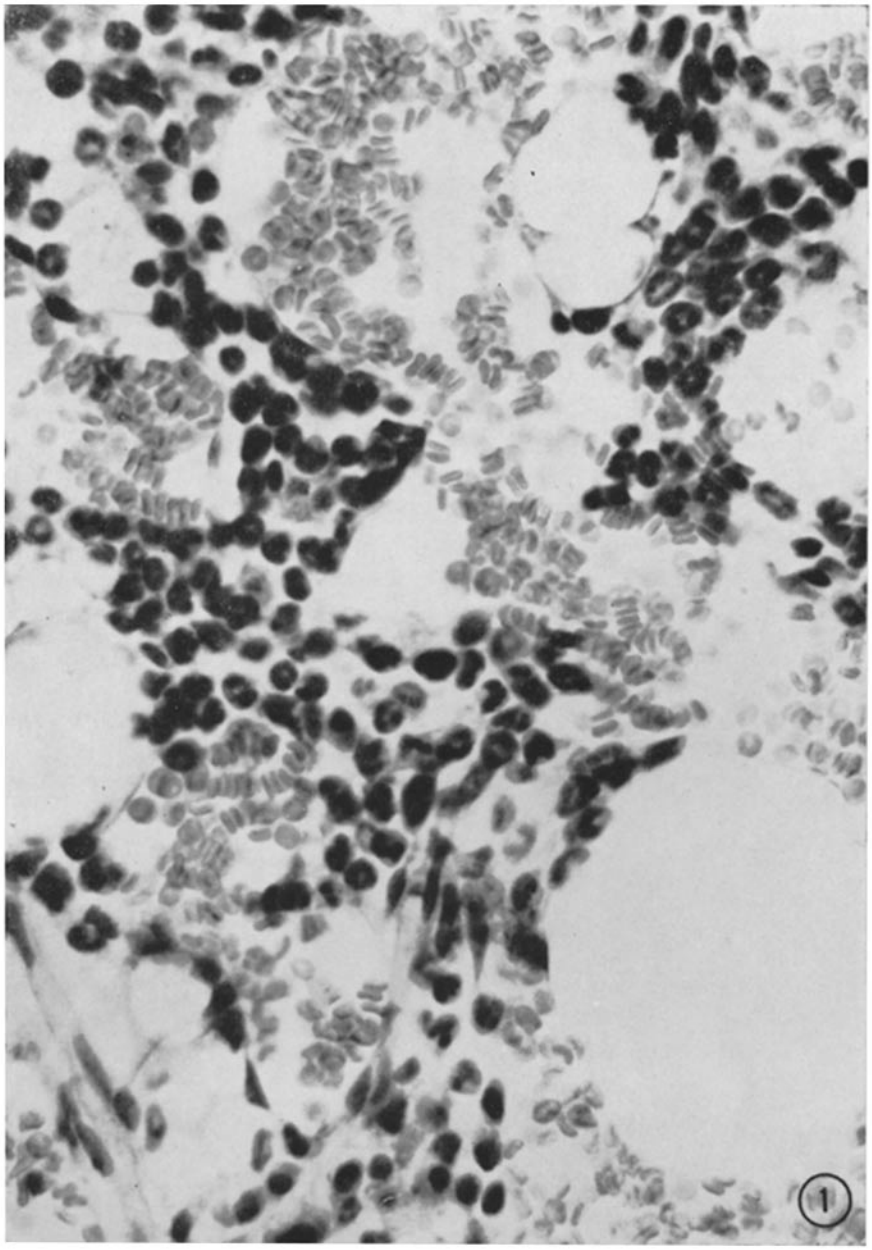
EXPLANATION OF PLATES

PLATE 30

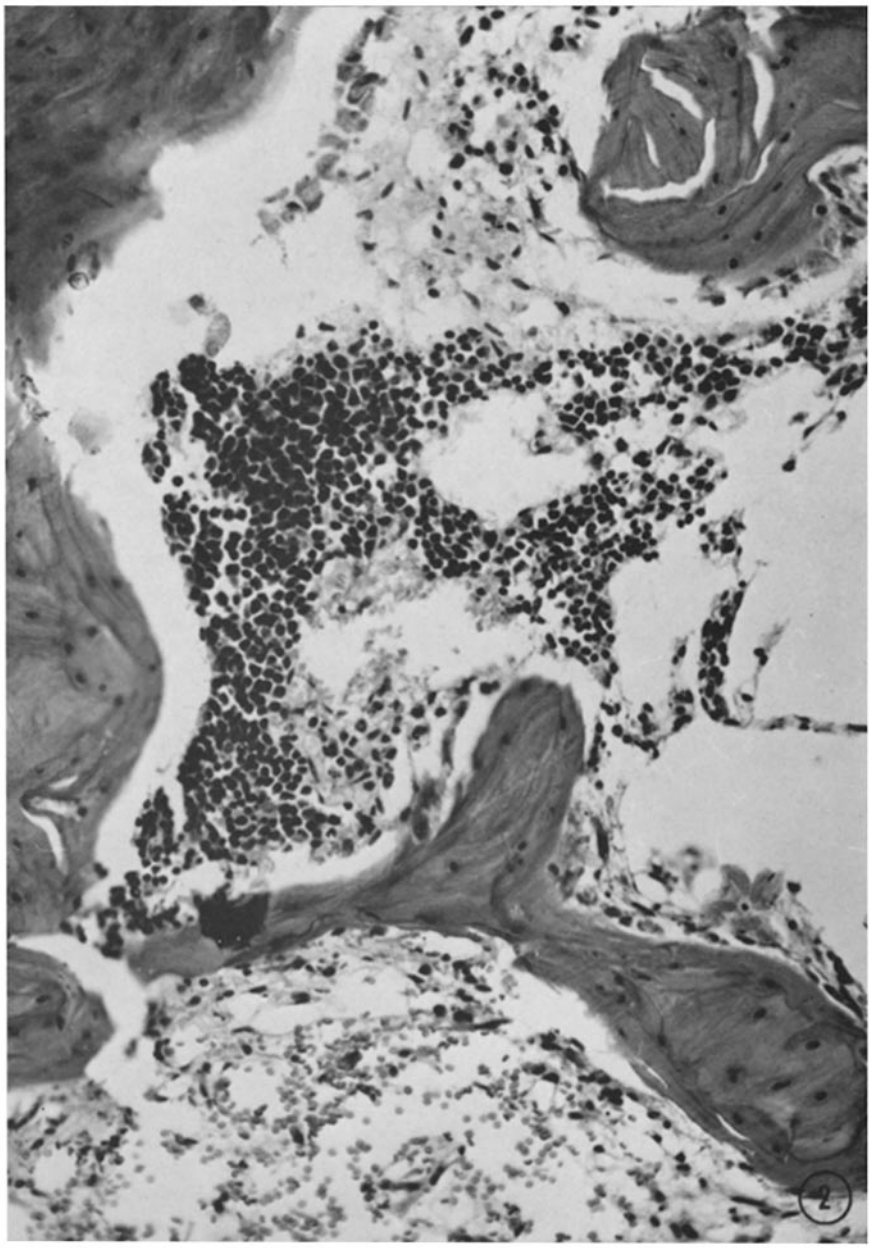
FIG. 1. An endogenous 7 day granulocytic colony in bone marrow of a 1000 R irradiated mouse. Approximately 90% of the area of this colony is included in the photograph. Hematoxylin and eosin. × 750.



(Wolf and Trentin: Hemopoietic colony studies. V)

PLATE 31

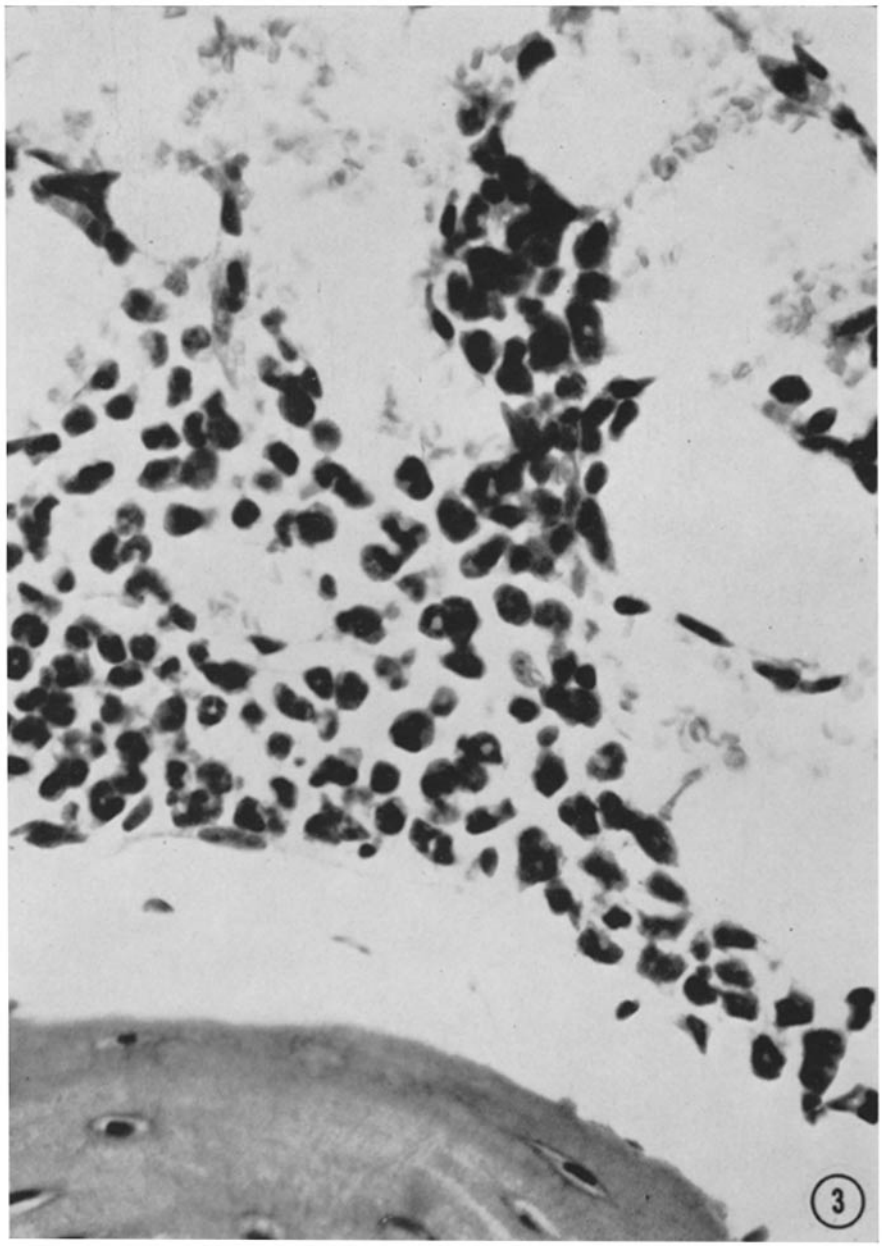
FIG. 2. A 7 day erythroid colony developing in the femur of a mouse which received 1000 R followed by 2×10^5 viable bone marrow cells intravenously. Hematoxylin and eosin. × 232.



(Wolf and Trentin: Hemopoietic colony studies. V)

PLATE 32

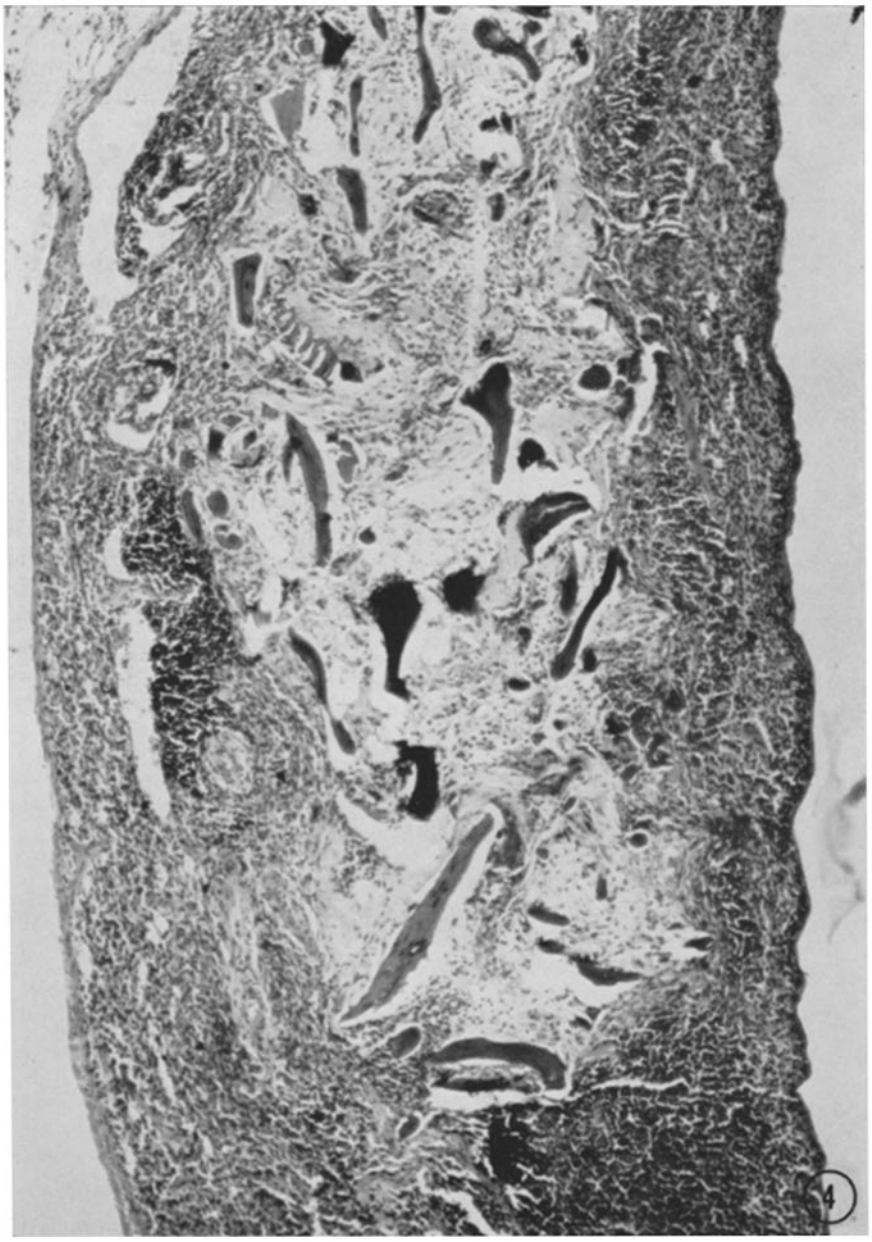
FIG. 3. A small 7 day granulocytic colony in bone marrow. This mouse received 1000 R plus marrow intravenously from an intermediate recipient (Table III). Approximately 90% of the area of this colony is included in the photograph. Hematoxylin and eosin. × 552.



(Wolf and Trentin: Hemopoietic colony studies. V)

PLATE 33

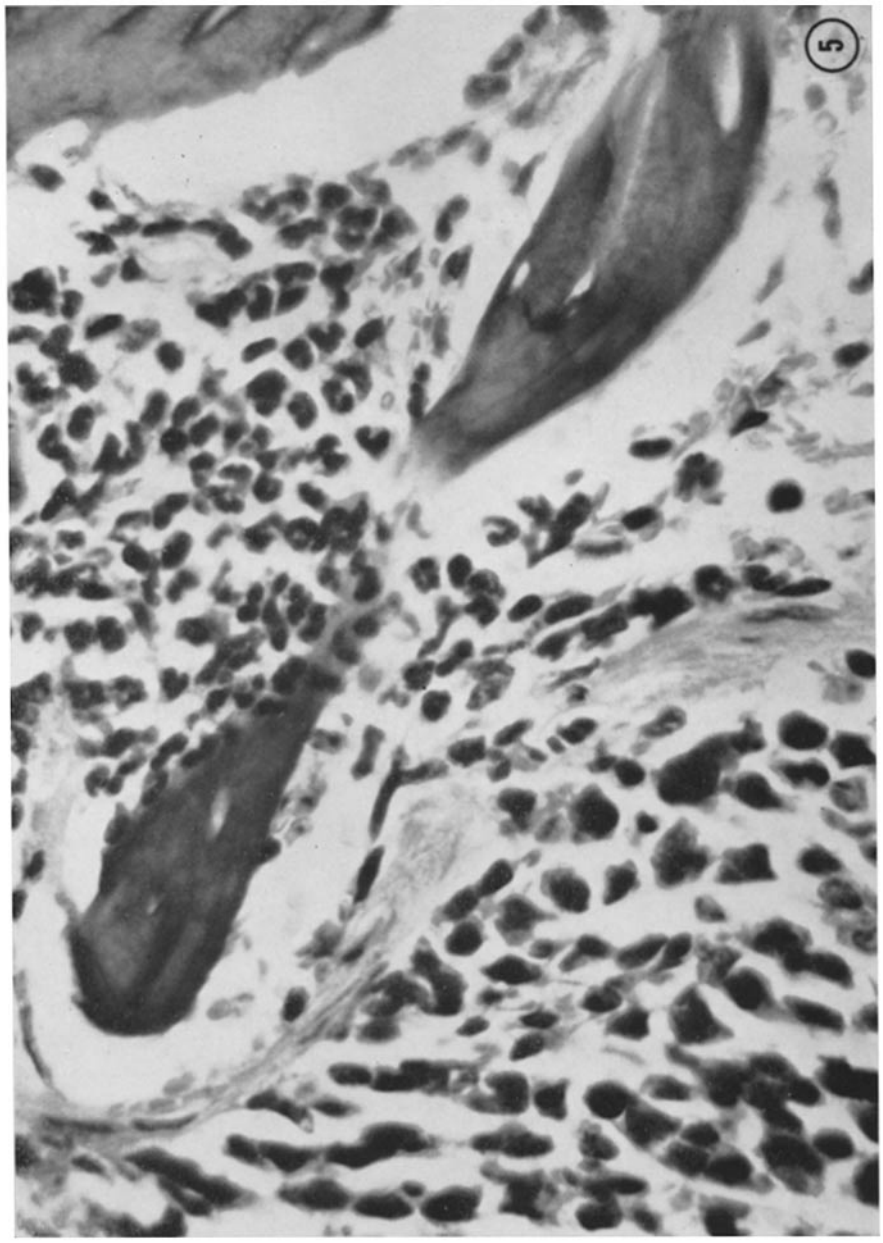
FIG. 4. Spleen containing a trocar implant of bone marrow stroma. Limits of the marrow stromal graft are evident from the bone spicules and less dense stroma of the marrow graft. The marrow stromal graft was obtained 18 to 24 hours after 1000 R whole body irradiation followed by intravenous injection of a bone marrow cell suspension. It was then trocar-implanted directly into the spleen of a 1000 R irradiated secondary recipient. The spleen was harvested 7 days later. The only source of CFU's in the secondary recipient of this experiment is the bone marrow stromal implant. Hemopoietic colonies are developing both in the marrow implant and in the adjacent spleen. Some colonies straddle the border between marrow implant and spleen. Hematoxylin and eosin. × 92.



(Wolf and Trentin: Hemopoietic colony studies. V)

PLATE 34

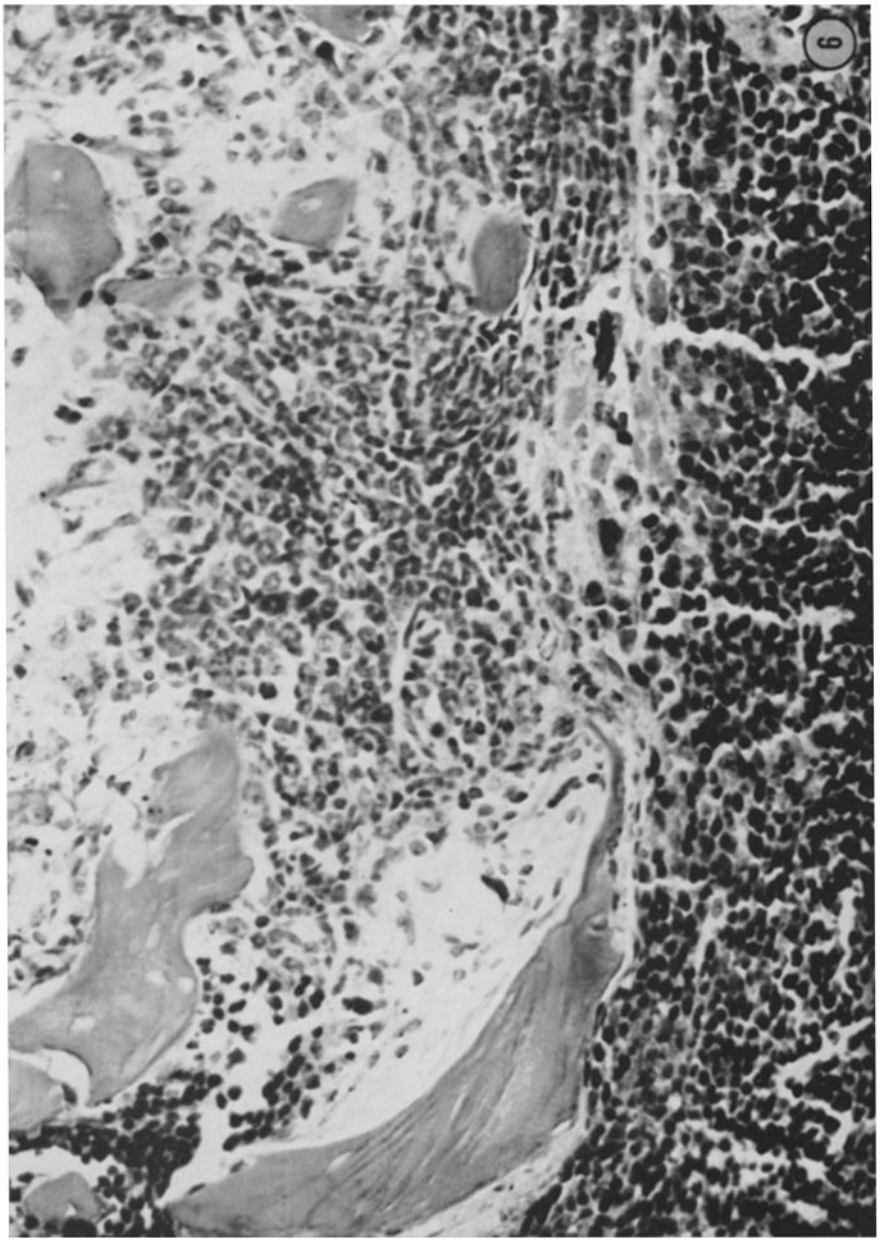
FIG. 5. Hemopoietic colony straddling the border of a bone marrow stromal implant (upper right) and adjacent spleen (lower left) of experiment of Table III and Fig. 4. Note granulopoietic cell types in marrow stroma, erythropoietic cell types in splenic stroma. CFU's originated in marrow implant. Hematoxylin and eosin. × 875



(Wolf and Trentin: Hemopoietic colony studies. V)

PLATE 35

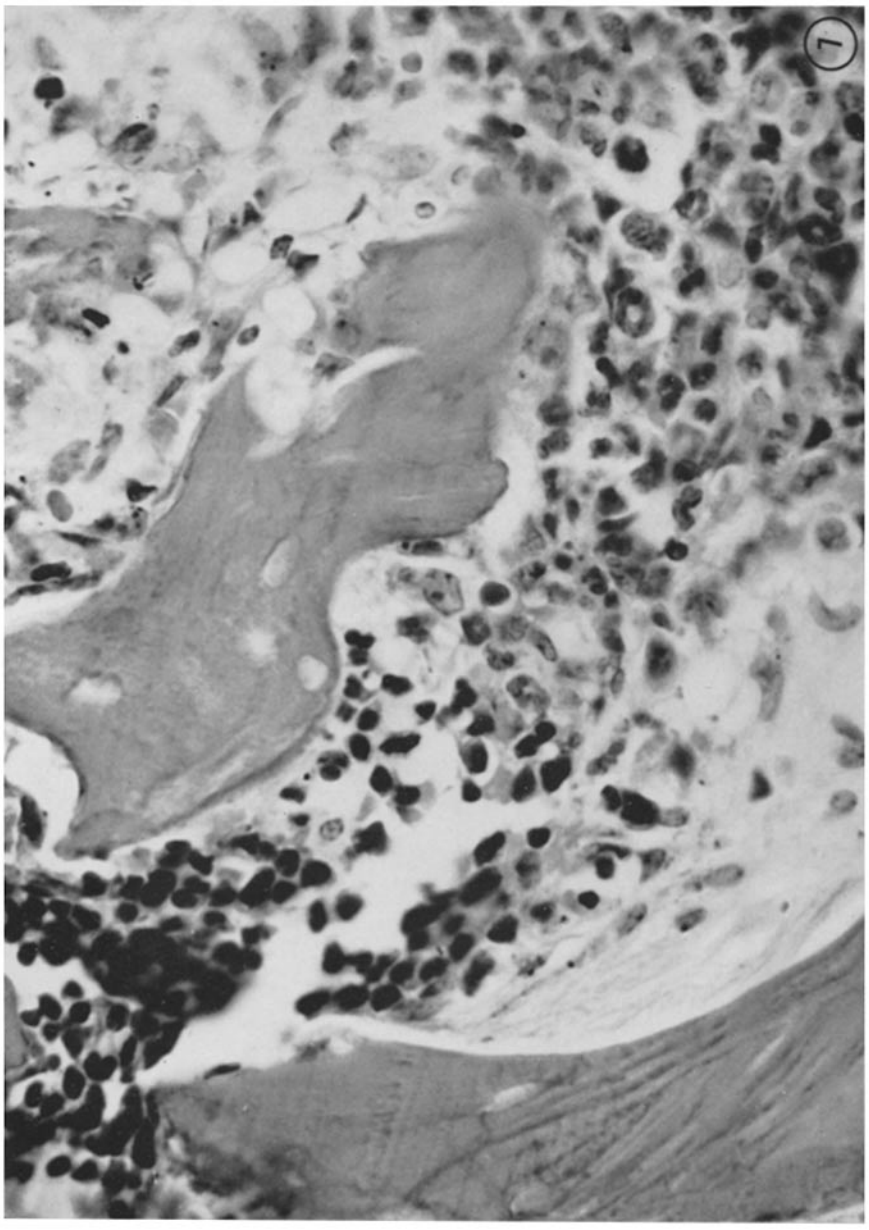
FIG. 6. A mixed hemopoietic colony straddling the border between bone marrow stromal implant and adjacent spleen. In this experiment (Table IV), 1000 R irradiated bone marrow stroma plus a 1 mm piece of unirradiated spleen were trocar implanted into the spleen of a 1000 R irradiated recipient. The only source of CFU's was the 1 mm unirradiated spleen graft. Note that the portion of the colony in the spleen stroma (lower and left) is erythropoietic, whereas the portion in the marrow stroma (upper and right) is granulopoietic. Hematoxylin and eosin. X 375.



(Wolf and Trentin: Hemopoietic colony studies. V)

PLATE 36

FIG. 7. An enlargement of Fig. 6 shows an area of cell type transition just inside the marrow implant perimeter. Erythroid cells are at top left, granuloid at bottom right. Hematoxylin and eosin. × 750.



(Wolf and Trentin: Hemopoietic colony studies. V)