leaves, sand, and silt (Table 2). At the collection site for this study *P. gracilis* was found on many types of substrates but most abundantly on rocks and in moss. Furthermore, it often inhabits headwaters of streams where eroding substrates prevail.

That *P. gracilis* mainly occupied a range of 4.5 to 22°C and had temperature preferences of 12.6°C and 14.8°C indicates it prefers comparatively cooler waters than *D. dorotocephala*. Chandler (1966) found *P. gracilis* at the headwaters of an Indiana stream which had year round cool temperatures and showed little annual variation, whereas *D. dorotocephala* occurred only downstream at warmer and less stable temperatures. Darlington and Chandler (1979) found some *P. gracilis* in Arkansas at 28°C, perhaps near the maximum temperature for this species. In previous temperature gradient experiments of other workers, *P. gracilis* aggregated at 0 to 10°C (Mast, 1903) and 0 to 9.5°C (Eddy and Gleim, 1932).

Phagocata velata exhibited the most clear cut preference for a particular substrate and the narrowest temperature range of the three species. Results indicate a highly significant preference (0.01 level) for rocks (Table 2), which is not in keeping with the observation that moss and silt were much more abundant than rocks at our collection site for *P. velata*.

In the temperature tests, *P. velata* preferred a range of 16 to 20°C. Although its temperature preference of 17.8°C is higher than *P. gracilis, P. velata* seems to be the most stenothermal of the three species. Field observations indicate that *P. velata* is often found in waters with small annual temperature fluctuation (Kenk, 1944; Darlington and Chandler, 1972; Kenk, 1974).

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TRANSPLANTABLE LEUKEMIA IN GUINEA PIGS

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ABSTRACT

Ten examples of leukemia in control or irradiated inbred guinea pigs were tested for transplantability. Nine were transplantable with 1 failure. Six of the original leukemias were classified as lymphatic and 4 as stem cell. As has been frequently observed in transplantable tumors, the latent period from transplant to death of the recipient from leukemia shortened with increasing numbers of transplant generations in those cases where the tumor was continued as a transplant for extended periods. The anatomical resemblance of lymphatic leukemia to the chronic disease in man was readily apparent. The stem cell leukemias closely resembled human acute leukemia.

INTRODUCTION

In an earlier paper we reviewed the evidence for radiation induction of leukemia in guinea pigs (Van Pelt and Congdon, 1972). The study was based on 48 cases observed in the experiments performed by Egon Lorenz and his colleagues at the National Cancer Institute during the 1940's and 1950's. Included in the 1972 study were the unusual and rapidly growing radiation induced stem cell leukemias of bone marrow origin that arose in fairly unique circumstances of protracted total-body irradiation. Also listed in that study were the 10 attempts to transplant the neoplasm to syngeneic hosts.

The present paper is a report of the 9 successful transplants that were established from these attempts. Some of the transplant lines (L2B, L2C) were used in cancer research by other laboratories as well as by those at the National Cancer Institute (Shevach et al, 1972; Stevenson et al, 1975).

Because leukemia in guinea pigs is less well known than in some other laboratory animals it seemed desirable to make a special record of these experiences with transplantation. The relative infrequency of spontaneous leukemia in this species together with the difficulty of inducing the leukemia reinforces the need for a special record. Perhaps more important is resemblance of chronic lymphatic leukemia and stem cell leukemia in guinea pigs to the leukemias seen in man, thus making another animal model available that could be useful in special circumstances.

Transplantation of guinea pig leukemia arising spontaneously in random bred animals was originally reviewed many years ago (Congdon and Lorenz, 1954). It was first attempted in a case reported by Miquez in 1918. The line was continued by Fischer and Kantor for 50 generations (1919). In 1926 Snijders began another series of transplants from a primary case which lasted 64 generations with 80 percent takes in random bred animals. Snijders characterized the tumor and found that transplantation could be effected by leukemic blood, thymus, lymph nodes, spleen, bone marrow, and pleural or peritoneal effusion.

The present work supplements the earlier reports and shows the availability of this animal for study of a disease that is a special problem in man.

MATERIALS

The 10 primary cases of lymphatic and stem cell leukemia that were transplanted are listed in Table 1 with identifying features. Autopsy records and slides as well as most of the experimental records on all of Egon Lorenz's guinea pig studies were kept at the Biology Division, Oak Ridge National Laboratory after his death. We reviewed this material there and extracted the information for the present report. All of the autopsy cards and slides have since been sent to the Registry of Experimental Cancers of the National Cancer Institute, Bethesda, Maryland. The available experimental records are deposited with the Archival Center for Radiation Biology at the University of Tennessee in Knoxville.

DESCRIPTION OF CASES

L 2 (1948-1954) was a lymphatic leukemia transplant series that was carried for [illegible] generations. The control male [illegible] was 104,800 and [illegible] to death.

The time between transplantation and death was 3¾ and 4½ months in 2 animals of the first transplant generation. It varied between 5 and 6 months to generation 9, and averaged 2 months from the 10th to the 17th. Transplantation was accomplished by the subcutaneous or intraperitoneal route and wide spread infiltration of lymphatic and many other organs occurred. One hundred percent takes were observed in Strain 2 recipients.

L 2B (1951-1955) was also a lymphatic leukemia that was carried for 93 generations according to our records. It has been subsequently studied by other investigators. A Strain 2 female (F 29) exposed to 2.2 R of gamma rays nightly (during an 8 hour period) to a total dose of 1768 R was the origin of this neoplasm. The animal died in the radiation field at 30¾ months with a terminal white blood cell count of 61,300. It was known to have been leukemic for 3 days.

The time between transplantation and death was 8¾ months in the first generation with a white blood cell count of 50,300. The time period was reduced to 8 days in the tenth generation and 14 in the twentieth, but stabilized at about 12 days for the remainder of the transplant series.

The route of transplantation was subcutaneous or intraperitoneal and there was widespread leukemic infiltration. One hundred percent takes were observed in Strain 2 recipients. Very high white blood cell counts were reported by Congdon and Lorenz (1954) in a study of the 10th generation of the series. They also suggested a predilection of leukemic infiltrates for sites of chronic inflammation.

L 2C (1953-1955), a lymphatic leukemia transplant series was carried for 31 generations in our records. It was still being

TABLE 1. *Summary of Guinea Pig Leukemia Transplant Experiments*[1]

Transplant Designation	Type of Leukemia	Result	No. of Transplant Generations	Animal Number	Group	Sex	Strain
L 2	Lymphatic	+Positive	17	A40	Control	M	2
L 2B	Lymphatic	+Positive	93	F29	F	F	2
L 2C	Lymphatic	+Positive	31	A83	Control	F	2
L 2D	Lymphatic	+Positive	1	H256	H-LIM+ SBM	F	2
L 2J	Stem Cell	+Positive	1	H299	H-LIM+ SBM	M	2
L 2L	Lymphatic	+Positive	?	G60	G-LIM	M	2
L 13	Lymphatic	+Positive	4	G36	G-LIM	F	13
L 13A	Stem Cell	−No Take	—	H278	H-LIM+ SBM	M	13
L 13B	Stem Cell	+Positive	11	H290	H-LIM+ SBM	M	13
L 13C	Stem Cell	+Positive	1	H276	H-LIM	M	13

1. Under animal number A, F, G, and H refer to control or radiation exposure levels. LIM means radiation exposure to a certain integral dose or level of anemia then removal from radiation field. +SBM refers to intravenous syngeneic bone marrow after removal from the radiation field (Van Pelt and Congdon 1972).

studied in 1972 by Shevach et al. The leukemia originated in an old (1229 days) Strain 2 control female (A 83). The terminal white blood cell count was 55,000 and leukemic blood was noted 1½ months before death.

The time between transplantation and death was 4 months in the first transplant animal. It was reduced to 23 days by the 10th generation, 17 days by the 20th and 15 days by the 31st. All transplants were made subcutaneously and there was widespread leukemic infiltration. In the 9th generation the white blood cell count reached 994,800. One hundred percent takes were reported in syngeneic hosts. Shevach et al. (1972) called L 2C a "B" cell leukemia.

L 2D (1955), a lymphatic leukemia, originated in a Strain 2 female (H 256) exposed to 8.8 R nightly (during an 8 hour period) then removed from the field and given syngeneic bone marrow. The accumulated dose was 940 R. Leukemia subsequently developed 1004 days following the irradiation. Age at death was 1199 days.

Spleen and lymph node tissue was transplanted into 5 pigs, 4 of which developed widespread leukemic infiltration. It took 7 months for the transplants to appear. The lesion was not retransplanted.

L 2J (1955) was a stem cell leukemia that appeared in a Strain 2 male (H 299) after limited radiation exposure and syngeneic bone marrow transplantation following cessation of the radiation. Intraperitoneal transplant of lymph node tissue to 2 female pigs did not take, but bone marrow and spleen to 3 males did with dissemination of the tumor. The time from transplant to death varied in these 3 animals from 3½ to 7 months.

H 299 was exposed to 8.8 R (during an 8 hour period nightly) to an accumulated dose of approximately 940 R. Age at death was 381 days. The pig survived 177 days after radiation.

L 2L (?-1961) was a transplant series originating in G 60 a Strain 2 male developing lymphatic leukemia. The "G" exposure level was 4.4 R (during an 8 hour nightly period). We had no records or slides on the transplant series but learned by correspondence that L 2L transplants were discontinued in 1961. G 60 was exposed to "G" for a limited period. It lived a total of 981 days and survived 529 days after cessation of the exposure. The average accumulated radiation exposure for the group was 1422 R.

L 13 (1948-1949) was a lymphatic leukemia transplant series lasting 4 generations before it was lost. The neoplasm originated in a Strain 13 female (G 36) that was exposed to 4.4 R gamma radiation nightly (during an 8 hour period) to a total dose of approximately 2556 R. It was then removed from the radiation field and developed leukemia at 31½ months of age (308 days after cessation of irradiation). G 36 had a terminal white blood cell count of 121,600. The leukemic blood was recognized 5 days before the terminal count.

In the 4 generations before the transplant was lost the time from transplant to death was 1½ months. There were 100 percent takes and extensive leukemic infiltration after subcutaneous transplantation.

L 13A was a stem cell leukemia arising in a Strain 13 male (H 278) that was exposed to 8.8 R nightly (during an 8 hour period) for a limited time, then removed from the radiation field and given syngeneic bone marrow. The radiation dose was 2473 R. There was no take on transplantation. Survival after cessation of radiation was 94 days.

L 13B (1954-1955) was lost after 11 transplant generations. It was a stem cell leukemia developing in a Strain 13 male (H 290) that had received syngeneic bone marrow after removal from the radiation field. The exposure was 8.8 R in the gamma field (for 8 hours nightly) to a dose of 1996 R. Age at death was 13¼ months and survival after irradiation 135 days.

The stem cell leukemia that subsequently appeared was transplanted subcutaneously and intraperitoneally. Time from transplantation to death was 2¼ months until the 10th generation, when it shortened to 1¼ months. There were 100 percent takes and leukemic infiltration of many tissues.

H 290 had the unusual "reactive change" in lymphatic tissue reported by Van Pelt and Congdon (1972). Some evidence of the "reactive change" in lymphatic tissue was observed in the transplant series too.

L 13C (1955) was one of the stem cell leukemias carried only for the first transplant generation—then lost. It originated in a male Strain 13 guinea pig (H 276) exposed to 8.8 R nightly (during an 8 hour period) to an accumulated dose of 1848 R. It was then removed from the radiation field and developed the stem cell leukemia in 745 days.

Bone marrow from H 276 was transplanted intraperitoneally into 5 Strain 13 male pigs. All transplants took and death of the recipients occurred between 2 and 2¾ months with leukemic infiltration of organs and tissues.

DISCUSSION

In 48 examples of leukemia arising from 629 control or irradiated guinea pigs, 20 were stem cell in type and 28 lymphatic leukemia (Van Pelt and Congdon, 1972). Nine out of 10 primary cases transplanted into syngeneic pigs were successful for both Strain 2 and 13 animals of control or irradiation groups. Some of the transplant series were carried for many generations and may still be available for cancer research.

Shortening of the time from transplant to death was noted in this study several times. As an example L 2B took 8¾ months to death in the first generation and stabilized around 12 days after the 20th to 93rd generation.

It is evident from other work done on these tumors, and especially that done by Jungeblut and Kodza (1960) and Nadel (1957), that each tumor, whether spontaneous or induced by radiation, maintains certain characteristics throughout its course. Thus some passages of L 2C to noninbred stock guinea pigs resulted in only local lymphosarcoma, but when this tumor was transplanted to Strain 2 animals, in which the tumor originated, a full spectrum of leukemia involvement was again obtained. In another instance (Jungeblut and Kodza, 1960), L 2C adapted or changed its pathologic presentation during the course of several generations so that there was extensive central nervous system involvement with a normal peripheral white blood cell count. When this tumor was returned to Strain 2 animals, the original type of L 2C leukemia was produced. Another lymphatic leukemia, L 2B on the other hand, maintained its usual pathology in noninbred animals, and after transplantation back to the original inbred Strain 2 (Nadel, 1957).

Nadel found that transplantation of L 2B to noninbred pigs under 10 days of age as 89% successful, whereas in animals of 3 to 7 weeks old there were only 31% takes, and after 8 weeks of age, transplantation was unsuccessful (Nadel, 1957). Using the same stock of noninbred guinea pigs, Jungeblut and Kodza (1960) were unable to detect the influence of age of the recipient on the success of L 2C transplants although this overall percentage of takes was low.

An historical account and further observations on the L 2C leukemia was recently published by Nadel (1977). In addition, a workshop covering many aspects of the L 2C leukemia transplant model was held in 1976 (Rhim and Green, 1977).

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SYNTHESIS OF THE RABBIT UTERINE PROTEIN, BLASTOKININ: EFFECT OF A PROTEIN-FREE DIET

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ABSTRACT

Rabbits cannot sustain pregnancy when deprived of dietary protein. These studies were an attempt to determine if a failure in uterine protein synthesis, particularly blastokinin, might account for the abortions.

Only half of the does bred within two days after being put on a protein-free diet, had normal embryos and normal levels of total uterine protein and of both blastokinin and the high molecular weight fraction in their uterine fluids on day 5 *post-coitum*. The others had low levels and/or failed to ovulate. After three days without protein our rabbits would not breed.

Because of the reported relationship between starvation and low circulatory progesterone levels, ovariectomized rabbits were deprived of dietary protein for two weeks and then subjected to a series of four daily injections of progesterone at concentrations normally known to induce high levels of synthesis of blastokinin. These animals however were unable to synthesize significant amounts of blastokinin and only low levels of other uterine proteins.

It is concluded that protein-deprived rabbits *may* produce enough uterine protein to support preimplantation embryogenesis but prolonged deprivation results in an inability to synthesize essential uterine proteins in response to exogenous progesterone.

INTRODUCTION

The reproductive success of mammals under conditions of starvation or severe nutritional deficiency varies greatly from one species to another. For example, most laboratory rodents cannot sustain pregnancy on a diet deficient in protein (Leathem 1966, Rattner et. al., 1978), while pigs carry their litters to term on diets devoid of or low in protein content (DeGeeter et. al., 1972, Anderson et. al., 1979). In our experience, rabbits also are unable to maintain pregnancy when starved for protein and we have been interested in how starvation affects their uterine function.

Recently, Murray et. al. (1979) showed that the uterine capacity for protein secretion is maintained in gilts even after 122 days on either a low-protein or a protein-free diet. We were encouraged to examine this same problem using rabbits because of their reproductive failure on such a diet (as noted) and because they synthesize the uterine protein, blastokinin (Krishman and Daniel, 1967) which is believed to be critical for survival of preimplantation stage embryos. This paper reports experiments designed to appraise the effect of a protein-free diet on the synthesis of blastokinin. It relates to earlier papers in this series which report changes in rate of blastokinin synthesis (Daniel and Booher, 1977), age dependency (Booher and Daniel, 1977) and the effect of light deprivation (Daniel, 1979).

METHODS AND MATERIALS

The experimental protocol is shown diagramatically in Figure 1 Twenty-four young adult New Zealand White female rabbits, divided into groups of 6 each, were used in this study: Three of the groups were maintained on a protein-free diet (ICN-Nutritional Bichemicals 904666) and the fourth group was fed a normal diet (Purina Checkers) containing 16% protein.

Group 1 animals were started on the protein-free diet (PFD) and then bred within two days thereafter and maintained on PFD for the anticipated gestation period (32 days) to confirm rabbits' inability to sustain pregnancy under this nutritional deficiency. Three of these same does were then returned to the normal diet (ND) for one month, bred again and retained on ND and the success of those pregnancies recorded.

Group 2 animals were also started on PFD, then bred as before and continued on the PFD until day five *post coitum*. At that time they were killed by cervical dislocation (Daniel and Boyce, 1978) and the uteri flushed with physiological saline solution into a watch glass to facilitate collection of embryos and uterine fluids. The blastocyst stage embryos were counted, measured and their normalcy appraised. The uterine flushings were kept cold and analysed by molecular seiving as described earlier (Daniel and Booher, 1977). The essential steps involved centrifugation to remove cellular debris, dialysis in Spectrapore membrane tubing overnight against distilled water, concentration with Aquacide (3-4 hours) and then used as the sample for filtration in a Sephadex G-200 column using citrate buffer at pH 7.4. Nine-drop fractions (0.5 ml) were collected with a LKB ultrorac fraction collector and each fraction (and the original sample) analysed for protein content by the method of Lowry et. al. (1951). These data were displayed graphically by plotting the optical density on the vertical axis against fraction