BNL 5815

STUDIES ON RADIATION INDUCED MAMMARY GLAND NEOPLASIA IN THE RAT

VII. The effects of fractionation and protraction of sub-lethal total body radiation.

Claire J. Shellabarger[1,2], Victor P. Bond, and Eugene P. Cronkite

Medical Department

Brookhaven National Laboratory[3]

Upton, L. I., New York

Submitted to: Radiation Research
No. of copies submitted: 2
No. of manuscript pages: 13
No. of tables: 1
No. of figures: 2

---

[1] Present address: Zoology Department and Medical School, University of Michigan, Ann Arbor, Michigan.

[2] Supported in part by N.C.I. Grant C-5496.

[3] Supported by the U. S. Atomic Energy Commission.

DISCLAIMER

Proposed running head:

Mammary Gland Neoplasia

Proofs to be sent to:

Dr. C. J. Shellabarger
Zoology Department and
Medical School
University of Michigan
Ann Arbor, Michigan

INTRODUCTION

It has been established that a single, sub-lethal, total body X- or gamma ray exposure of the young, female, Sprague-Dawley rat results in the relatively early appearance of mammary neoplasia (1-3). Since this neoplastic response of mammary tissue appears to be related to the dose in a linear fashion, within the limits of 25r and 400r (4), the dose that produced the maximum incidence of rats with mammary neoplasms was selected to study the neoplastic potential of this amount of radiation given either as a single dose or as multiple doses. This report presents data that indicate that the neoplastic potential of sub-lethal, total body radiation is not changed by fractionating and protracting the exposure within the limits studied.

METHODS

One hundred and twenty-four, litter-mate, female, Sprague-Dawley rats were assigned to four groups of 31 rats each so that approximately equal numbers of litter-mates appeared in each group. They were exposed as follows: One group received 400r on the 40th day of age; another group received 400r given in five equal, daily doses of 80r on the 38th through the 42nd days of age; one group received 400r given in 18 equal, daily doses of 22.2r given on the 32nd through the 49th days of age; and one group was reserved as non-exposed controls. All radiation exposures were given at the same dose rate, approximately 60r per minute, and the same total dose of 250 kvp X-rays was given to all of the exposed groups, using a therapy X-ray machine operated at 30 ma with 0.5 mm Cu and 1.0 mm Al filters with a TSD of 60 cm. The rats were exposed in groups of 7 or 8 in lucite boxes placed on 1 inch of masonite on an aluminum rotating table. The dose was measured in air using at least a half scale reading on a 100r Victoreen Chamber under the

same scatter conditions as in the actual exposure(s) at a point corresponding to the midpoint of the animals.

The rats were studied for a period of one year after exposure, taken as the 40th day of age. During this time they were housed 7 or 8 rats per cage, at 72° ± 3° F, and given commercial rat chow and water ad libitum. The rats were examined frequently for the presence of neoplasia of the breast. When such a neoplasm was found, it was allowed to grow until it reached a 2-3 cm size at which time it was removed and the rat returned to the experiment. One year after exposure all rats still alive were killed and examined for evidence of gross neoplasia. Only after histological verification were the neoplasms recorded as being neoplasms and tabulated as such. All mammary neoplasms were tabulated as months post-exposure when first found. If mammary neoplasms were found at different sites they were recorded as successive neoplasms; if they occurred at sites of previous neoplasms they were called re-occurrences and not tabulated. Each mammary neoplasm was given a classification of either adenocarcinoma, adenofibroma, fibroadenoma, or fibrosarcoma. Photomicrographs of these have been published previously (1,5) and are not included in this report.

RESULTS

The number of rats exhibiting mammary neoplasms and the total number of mammary neoplasms was higher in the exposed groups than in the non-exposed group (Table 1). Although the incidence of rats with mammary neoplasia ranged from 58 to 77 per cent among the exposed groups, there were no statistically significant differences, as judged by the chi-square test (6), among the 3 exposed groups. Just how different the response of the fractionated and protracted groups would have had to be in order that their response would have been statistically different from the response of the group that received 400r in a single exposure is indicated by the shaded portion of Figure 1.

The total number of mammary neoplasms observed in the 3 exposed groups ranged from 28 to 43 and this difference was not considered to be of significance (Table 1, Figure 2). Supporting the conclusion that fractionation and protraction of the 400 r dose produced little change in the mammary neoplastic response was the fact that no regular progression of responses was noted with increasing protraction of smaller and smaller doses. In fact, the response noted in the 22.2r x 18 group was usually closer to the response found when the 400r dose was given as a single exposure than were the results when the 400r dose was given in 5 fractions.

It was clear that the exposed groups exhibited mammary neoplasia much sooner (at a younger age) than did the non-exposed animals, both on a percentage basis of rats with mammary neoplasms and on the basis of the total number of mammary neoplasms (Figures 1-2). There were no large differences noted among the exposed groups in these responses and in fact, the period of 2 months between radiation exposure (taken as the 40th day of age in all cases) and appearance of the first mammary neoplasm was the same in all exposed groups. When the incidence of rats with mammary neoplasia was corrected on a life table basis (7) by taking into account the number of animals at risk at any monthly period no differences were found by this method of analysis as compared to the analysis of the raw data. Here again, there was no progression of response noted as fractionation increased, nor was there any correlation of survival rate with the type of exposure.

All three of the mammary neoplasms that occurred in 3 of the non-exposed rats were classified as either adenofibromas or fibroadenomas. On the other hand, among the exposed groups there were 103 mammary neoplasms found in 61 rats and 72 of these mammary neoplasms were classified as either adenofibromas or fibroadenomas, 25 were classified as adenocarcinomas, 2 were classified as fibrosarcomas, and 4 did not fall into any of these

classifications. There were no large differences in types of mammary neoplasms among the 3 exposed groups that could be correlated with fractionation and protraction.

The incidence of neoplasia of non-mammary tissue origin approximated 6 per cent in the exposed groups and 2 per cent in the non-exposed group. This small difference was not analyzed further.

DISCUSSION

It is clear from the data here reported and from previous reports (1-4) that the young, female, Sprague-Dawley rat shows a rapid and relatively large mammary neoplastic response to sub-lethal, total body, X-ray exposure. The situation studied in the present experiment revolves around the question, "If the same total dose is sustained, but if this dose is fractionated and protracted, will the mammary neoplastic response be changed?" The present experiment involves only a modest fractionation of the dose spread over a relatively short period of time. The protraction was limited for 2 reasons. First, it is not easy to analyze the results if the radiation exposure is spread over long periods of time because of the difficulty of selecting a time period to be used as the reference point to begin the tabulation of the neoplastic response. Mathematical ways to circumvent this difficulty are available, but these methods are complex and it was decided to purposely limit the exposure to the 40th day of age plus or minus 9 days for a total of 18 days so that all measurements of neoplastic responses could be referred back to the mid-point of this exposure period. Thus, all mammary neoplasms were tabulated as occurring in the nearest month following the 40th day of age although the actual exposures took place at extremes of the 32nd to the 49th day of age. On this basis, there appeared to be no effect of a small fractionation and protraction procedure, as compared to a single exposure. There are insufficient data, however, to allow

predictions of how the neoplastic response might vary if the same total dose was spread over longer exposure periods.

A second limitation was placed upon the extent of protraction in the present experiment because of the possibility that the sensitivity of the neoplastic response might change with the age of the animal being exposed. Although there is no evidence for changes of sensitivity with age for this neoplastic response in this strain of rat, nonetheless the period of exposure, in terms of age of the animals, was restricted to the period between 32 and 49 days of age. Similarly, since it is known that the incidence of mammary neoplasia increases with age in the nonexposed female rat (3) this incidence of neoplasia not due to radiation could confuse the interpretation of the data from exposed animals. This "aging" complication was obviated by studying only young animals at an age before the control incidence became appreciable. Here, again, the present finding of a lack of effect of a limited protraction regimen can not be used to predict what might happen to neoplasia incidence over long, i.e., life time, fractionated exposures.

Another limitation on the extent of fractionation and protraction was imposed because it is likely that fractionated and protracted exposures produce less severe effects on life shortening than do single exposures (8). Thus, if the procedures of fractionation and protraction were to act to allow the animals to live longer than those animals exposed to the same total dose given as a single exposure, a larger neoplastic response of the remaining larger group of older animals might be expected. In the present experiment, the mortality rates were not dissimilar in all exposed groups and a "life-sparing" effect cannot account for the similar mammary neoplastic response noted in animals exposed to the same total dose given as a single exposure or as multiple exposures.

The direct comparison of fractionated and protracted exposures with a single exposure of the same total dose of total body radiation on the induction of neoplasia in the rat has been made only infrequently (9). Lamson, et al (10) report that when rats were exposed to total body radiation under hypoxic conditions and studied until death, 9 of 22 rats that survived a single dose of 800r exhibited neoplasia largely of mammary origin while 10 of 11 rats exposed to 2 doses of 400r exhibited mammary neoplasia. Although these investigators suggest that total body irradiation delivered as a fractionated exposure may be a more potent stimulus to early appearance of neoplasia than a single exposure, they also point out that the incidence of nephrosclerosis observed in these rats may complicate the interpretation. Lamson, Meek and Bennett (10) further suggest that the finding of an apparent larger neoplastic response in the case of fractionation than in the case of single exposure argues against the single-hit theory of direct irradiation effects. If, however, the suggestion of Bond, et al (11) is correct that carcinogenesis induced by radiation is more than a one-step process, then the suggestion of Lamson, et al that their results negate the single-hit theory of neoplasia induction needs further examination. Certainly, it is true that the animals of Lamson, et al received the same dose to the breast tissue whether given as a single or as a fractionated exposure but it is reasonable to assume that the effect of single and fractionated exposures on ovarian function might differ. Thence, the different hormonal stimulation that the mammary tissue received under different exposure conditions might well account for the difference in neoplasia response following single or fractionated exposures, (2,11). It should be pointed out also that while the doses were fractionated in the present experiments, the instantaneous dose rate was essentially the same in all exposures, 60r/min. Thus considerations applicable to lower dose rates, or possible recovery over a matter of hours (12), do not necessarily apply.

Other comparisons of fractionated and protracted exposures on neoplasia induction include the following. Cole, et al (13) has studied the effects of 690r of X-ray in either single or divided doses on the induction of lymphoma-leukemia and ovarian adenoma in mice. This study was complicated by the fact that the irradiated animals did not live as long as their nonexposed controls. The incidence of lymphoma-leukemia was no higher in the irradiated animals than in the non-irradiated animals, and the incidence of ovarian adenoma showed no regular change with increasing fractionation. Brues, et al (14) and Kaplan, et al (15) have shown that to induce leukemia in the mouse an optimal fractionation and spacing of exposures was more effective than the same dose given as a single exposure. It would appear from subsequent experiments of Kaplan and Brown (16) that the induction of mouse leukemia is by an indirect mechanism and thus may represent, as pointed out below, a special rather than a general case of radiation carcinogenesis. While not strictly total body radiation experiments, the reports of Henshaw, et al (17) and Glucksmann (18) on the induction of skin tumors by beta or electron beam exposures do not provide data suitable for comparison of tumor response at comparable total doses. Chronic radiation exposure studies usually involve the comparison of large and different total doses, and in addition the concept of "wasted radiation" may make any direct comparison of the neoplastic responses at equal dose levels impossible (19).

The finding that fractionation and protraction did not reduce the neoplastic potential of total body radiation, in the present study, has important implications concerning the mechanism of the induction of mammary neoplasia in the rat. All of the reports concerned with the induction of mammary neoplasia in the rat presently available are consistent with a direct mechanism that somewhat resembles the genetic changes induced by radiation.

In studies with partial body exposures the mammary neoplastic response appears to be a direct effect of the radiation exposure since almost all of the neoplasms appear in the exposed area and not in the nonexposed area (11). Further, there appears to be a linear relationship between dose and response within the limits studied (4). And now, with limited fractionation and protraction procedures, the mammary neoplastic response appears to take on the characteristics of a cummulative response. The last two findings need further substantiation and experiments are in progress to re-evaluate the effects of increasing doses and more widely spaced fractionation and protraction. However, at the present time, all of the results obtained with mammary neoplasia of the rat are not inconsistent with a non-threshold, direct mechanism for the "primary event" leading to neoplasia induction by sub-lethal, total body radiation.

Which is the more general phenomenon, the induction of mammary neoplasms in the rat by a presumed direct mechanism that appears to be dependent upon the total dose and appears to be independent of the dosage schedule, or the indirect mechanism of mouse thymoma-leukemia induction that depends upon a rigid dose spacing schedule? The question cannot be answered with any great degree of confidence at the present time. Kaplan (20) has chosen the indirect mechanism as being the more likely to represent the general situation, as has Brues (21). The main argument leveled against the direct mechanism seems to stem from the absence of linear dose response relationships. Mole (22) has pointed out that the failure to observe a linear dose response relationship does not prove that a linear dose response relationship does not exist, especially since it has been pointed out that the absence of an obvious linear dose response relationship may be predicted if a "two step" mechanism of radiation carcinogenesis is operative (11). It should be pointed out that since there is now at least one situation--the induction of mammary neoplasia in the rat--that appears to fit the criteria for a direct mechanism underlying the

production of radiation carcinogenesis, this direct mechanism should not be disregarded too hastily as having a general application in radiation carcinogenesis.

SUMMARY

Three groups of 31 female, Sprague-Dawley rats were given a total body exposure of 400r of 250 kvp X-rays, according to the following schedule: 22.2r daily from the 32nd through the 49th day of age; 80r daily from the 38th through the 42nd day of age; 400r on the 40th day of age; or no exposure. Thefinal cumulative incidence of rats with one or more histologically verified neoplasms of mammary origin at 12 months after the 40th day of age was: 22.2r x 18, 61%; 80r x 5, 58%; 400r x 1, 77%; control, 10%. As judged by the chi-square test, the response was not different among the 3 exposed groups but the incidence of each exposed group was greater than that of the control group. These results were interpreted to mean that the neoplastic response of rat mammary tissue to total body radiation is independent of fractionation and protraction within the limits of the dosage schedules studied. Since the experiment was terminated at one year after exposure, any effects on shortened life span were obviated and nothing was learned about the final or life span incidence of mammary neoplasms. The incidence of non-mammary tissue neoplasia did not exceed 6% in any group; thus no conclusions could be drawn about the effect of fractionation and protraction on this response. It was pointed out that the failure of fractionation and protraction to reduce the neoplastic potential of sub-lethal total body radiation, taken together with previously published reports suggests that the neoplastic response of mammary tissue of the rat is not inconsistent with a non-threshold, direct mechanism for the primary event leading to neoplasia induction by sub-lethal, total body radiation.

ACKNOWLEDGEMENTS

The authors are greatly indebted to Doctors S. W. Lippincott and G. E. Aponte who reviewed all microscopic sections.

REFERENCES

1. C. J. Shellabarger, E. P. Cronkite, V. P. Bond, and S. W. Lippincott, The occurrence of mammary tumors in the rat after sublethal whole-body irradiation. Radiation Research 6, 501-512 (1957).

2. E. P. Cronkite, C. J. Shellabarger, V. P. Bond, and S. W. Lippincott, Studies on radiation-induced mammary gland neoplasia in the rat. I. The role of the ovary in the neoplastic response of breast tissue to total- or partial-body X-irradiation. Radiation Research 12, 81-93 (1960).

3. C. J. Shellabarger, V. P. Bond, and E. P. Cronkite, Studies on radiation-induced mammary gland neoplasia in the rat. IV. The response of females to a single dose of sublethal total-body gamma radiation as studied until the first appearance of breast neoplasia or death of the animals. Radiation Research 13, 242-249 (1960).

4. V. P. Bond, E. P. Cronkite, S. W. Lippincott, and C. J. Shellabarger, Studies on radiation-induced mammary gland neoplasia in the rat. III. Relation of the neoplastic response to dose of total-body radiation. Radiation Research 12, 276-285 (1960.)

5. C. J. Shellabarger, S. W. Lippincott, E. P. Cronkite, and V. P. Bond, Studies on radiation-induced mammary gland neoplasia in the rat. II. The response of castrate and intact male rats to 400 r of total-body irradiation. Radiation Research 12, 94-102 (1960).

6. D. Mainland and I. M. Murray, Tables for use in fourfold contingency tests. Science 116, 591-594 (1952).

7. R. Sacks, Life table technique in the analysis of response-time data from laboratory experiments on animals. Toxicol. and Appl. Pharmacol. 1, 119-134 (1959).

8. Z. M. Bacq and P. Alexander, Fundamentals of Radiobiology. Pergamon Press, New York, 1961, Chapter 17.

9. A. Glucksman, L. F. Lamerton, and M. V. Mayneord, Cancer (R. W. Raven, Ed.), Vol. 1, p. 497, Butterworths Publications, London, 1957.

10. B. G. Lamson, R. A. Meek, and L. R. Bennett, Late effects of total-body roentgen irradiation. II. The influence of fractionated and single radiation doses on the incidence of tumors, nephrosclerosis and adrenal vacuolation in Wistar rats during various periods of post-irradiation survival. Arch. Path., 505-521, 1957.

11. V. P. Bond, C. J. Shellabarger, E. P. Cronkite, and T. M. Fliedner, Studies on radiation-induced mammary gland neoplasia in the rat. V. Induction by localized irradiation. Radiation Research 13, 318-328 (1960).

12. M. M. Elkind, and H. Sutton, Radiation response of mammalian cells grown in culture I. Repair of X-ray damage in surviving chinese hamster cells. Radiation Research 13, 556-593,(1960).

13. L. J. Cole, P. C. Nowell, and J. S. Arnold, Late effects of X-radiation. The influence of dose fractionation on life span, leukemia and nephrosclerosis in mice. Radiation Research 12, 173-185 (1960).

14. A. Brues, G. A. Sacher, M. P. Finkel, and H. Lisco, Comparative carcinogenetic effects by x-radiation and P^{32}. Cancer Res. 9, 545 (1949)Abstract.

15. H. S. Kaplan and M. B. Brown, A quantitative dose-response study of lymphoid tumor development in irradiated C_{57} black mice. J. Natl. Cancer Inst. 13, 185-208 (1952).

16. H. S. Kaplan and M. B. Brown, Development of lymphoid tumors in non-irradiated thymic grafts in thymectomized irradiated mice. Science 119, 439-440 (1954).

17. P. S. Henshaw, R. S. Snider and E. F. Riley, Aberrant tissue developments in rats exposed to beta rays. Radiology 52, 401-414 (1949).

18. A. Glucksmann, Carcinogenesis of skin tumors induced by radiation. British Med. Bull. 14, 178-180 (1958).

19. R. H. Mole, On wasted radiation and the interpretation of experiments with chronic irradiation. J. Natl. Cancer Inst. 15, 907-914 (1955).

20. H. S. Kaplan, Some implication of indirect induction mechanisms in carcinogenesis. A review. Cancer Res. 19, 791-803 (1959).

21. A. M. Brues, Critique of the linear theory of carcinogenesis, Science 128, 693-699 (1958).

22. R. H. Mole, The dose-response relationship in radiation carcinogenesis. British Med. Bull. 14, 184-189 (1958).

TABLE I

The number of rats, the number of rats surviving 12 months (post exposure and/or the 40th day of age), the number and percentage of rats with mammary neoplasms, and the total number and pathological types of the mammary neoplasms for each experimental group.

Treatment	Number of Rats			Per cent of rats with mammary neoplasms		Number and type of mammary neoplasms				
	Starting	12 month survivors	with mammary neoplasms	Raw	Corrected *	Total	AC	FA or AF	FS	M or O
None	31	22	3	10	13	3		3		
400 r x 1	31	23	24	77	82	43	11	29	2	1
80 r x 5	31	17	19	61	79	32	5	25		2
22.2 r x 18	31	15	18	58	71	28	9	18		1

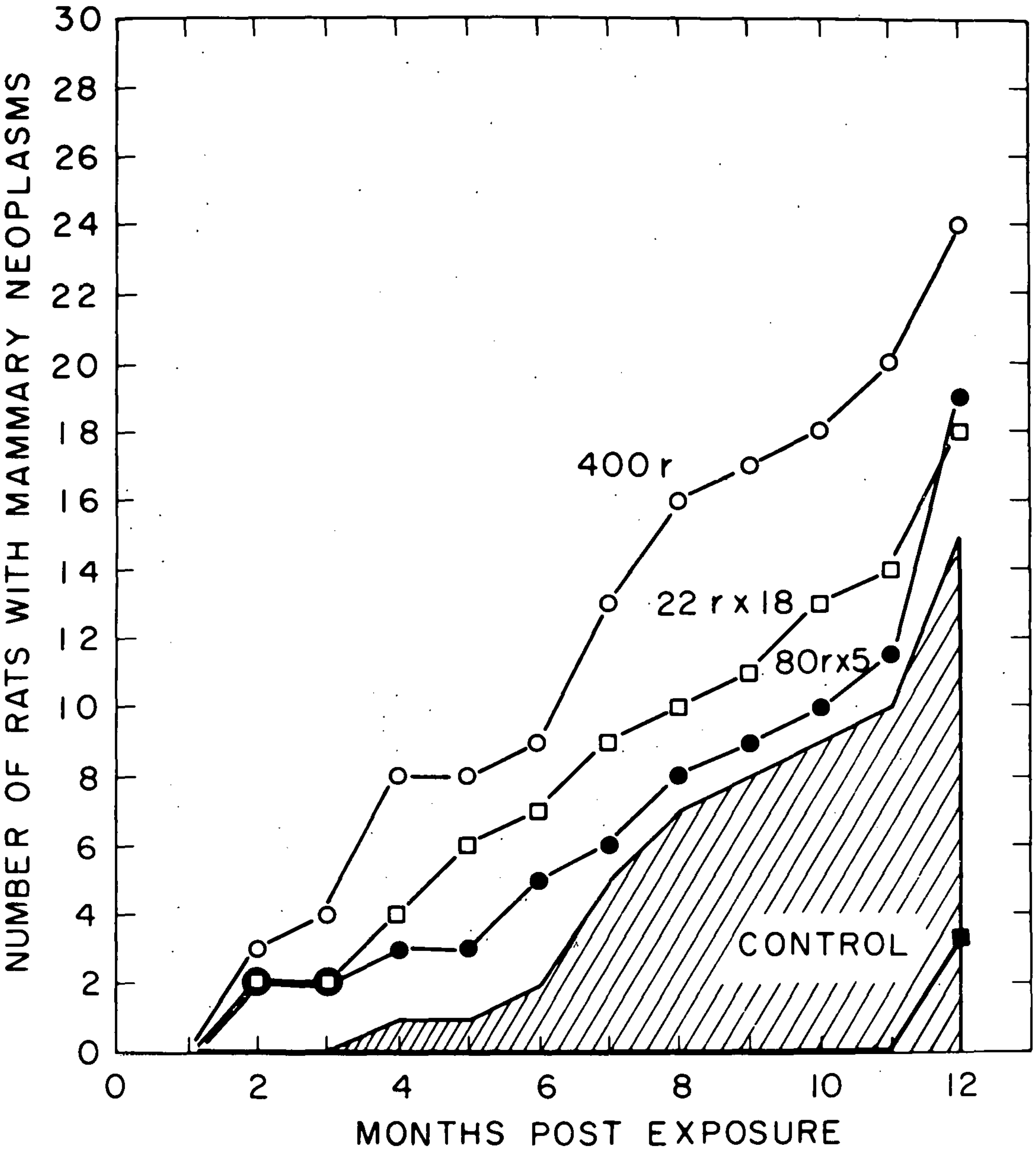
FS, fibrosarcoma
AC, adenocarcinoma
AF, adenofibroma
FA, fibroadenoma
M, Mixed
O, Other

* Corrected by the life table technique (7)

FIGURE LEGEND

Figure 1. Number of rats with one or more mammary neoplasms plotted against the months post-exposure and/or the 40th day of age for 3 exposed groups and one non-exposed group. The shaded portion indicates the minimum contrast need to be statistically different, at the 5 per cent level of confidence, from the response of the 400 r group, as judged by the chi-square test (6). Starting number, 31 rats per group. Neg. No. 11-433-61.

Figure 2. Number of mammary neoplasms plotted against the months post-exposure and/or the 40th day of age for each experiment group. Starting number of rats, 31 per group. Neg. No. 4-882-60.



BNL Neg No. 11 1133-61 Fig 1