Cytotoxic Effect of Leukemic Guinea Pig Plasma on Normal Guinea Pig Spleen Cell Tissue Cultures.* (25945)

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Congdon and Lorenz(1) described occurrence of acute lymphocytic leukemia in an inbred strain (#2) of guinea pigs. The disease was characterized by widespread leukemic involvement of the entire hematopoietic system, with high blast cell counts in the peripheral blood, and could be transmitted by transfer of tumor tissue. We have previously shown that the disease can be reproduced in first generation hybrids, originating from crossmating susceptible strain #2 with resistant Hartley guinea pigs(2), and that transmission succeeds equally well with leukemic plasma or with leukemic spleen extract(3). This paper reports a cytotoxic effect which may be observed when plasma from leukemic guinea pigs is added to normal guinea pig spleen cells growing in primary tissue culture.

Materials and methods. Leukemic plasma was prepared from centrifuged heparinized blood; the blood was drawn from hybrid guinea pigs (strain #2 x Hartley, F_1), inoculated with leukemia L_2C, at height of the disease. Two cycles of cold centrifugation at 5500 rpm for 20 minutes were used to separate plasma from cellular elements of the blood. Plasma prepared by the same method from uninoculated hybrid guinea pigs served as control. Tissue cultures were prepared in square, rubber-stoppered flasks from finely minced, embryonic or adult normal guinea pig spleens (Hartley strain); the cells were suspended in medium 199 which contained 15% normal horse serum, human serum or guinea pig serum (plus antibiotics). Flasks were incubated at 37°C and fluid was replaced thrice weekly with fresh medium. When continuous monolayers of fibroblasts and epithelial-like cells had developed—usually after 12 to 20 days—freshly harvested leukemic plasma was added in a volume of 0.5 ml to 10 ml of tissue culture fluid. The flasks were reincubated and cell sheets were examined microscopically during the next few days for morphological changes. It goes without saying that rigid sterility was maintained throughout all experiments.

Results. Twenty different samples of leukemic plasma were tested as described above. Fourteen samples produced a definite destructive effect which was noted after 24 to 72 hr contact between leukemic plasma and tissue culture cells. This effect began with increased granularity, vacuolization and cutting off of groups of spindle cells; in later stages, this was followed by more or less complete disintegration of both fibroblastic and epithelial-like cells, terminating with exfoliation of the sheet when the effect was maximal (Fig. 1-4). A similar effect was noted in one experiment with leukemic serum and in 3 out of 4 experiments in which twice centrifuged leukemic spleen extract was used as inoculum. Normal guinea pig plasma, or serum, produced no deleterious effects on the cells. Moreover, when leukemic plasma which proved fully active on normal guinea pig spleen cell cultures was added to established cell lines of leukemic guinea pig spleen cell, HeLa cell or human amnion cell cultures or to primary whole embryonic mouse cell cultures, no cytotoxic effects were seen. A certain degree of specificity of the phenomenon is therefore apparent.

The cytotoxic factor in leukemic serum was filtrable through a Millipore membrane of 0.45 μ porosity and was thermostabile up to one half hour heating at 65°C. Titration of leukemic plasma indicated a maximal potency up to a 1:500 dilution. Attempts were made in 5 experiments to maintain the cytotoxic effect over serial tissue culture passages; however, in no case did transfers succeed beyond the quantitative limits of potency of the original inoculum. The results were no different

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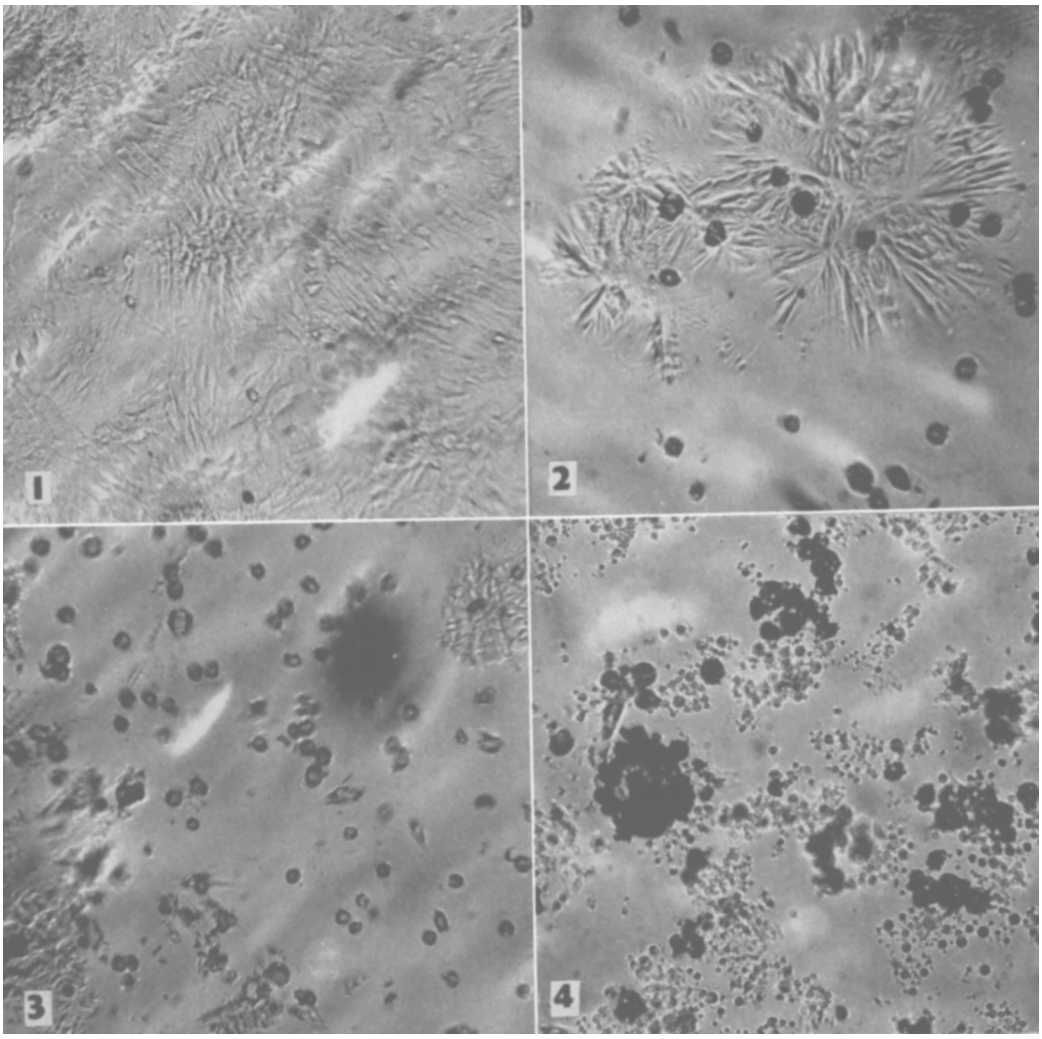

FIG. 1. Normal adult guinea pig spleen cells growing in primary tissue culture (control).
FIG. 2. Early cytotoxic effect produced by leukemic guinea pig plasma.
FIG. 3. More advanced cytotoxic effects produced by leukemic guinea pig plasma.
FIG. 4. Maximum cytotoxic effect produced by leukemic guinea pig plasma.

when tissue cultures were used which had been prepared from strain #2 embryonic guinea pig spleens suspended in strain #2 serum. In 4 different experiments susceptible guinea pigs were injected with tissue culture material representing cells or fluids from flasks with cytotoxicity. None of 6 animals in this group developed clinical signs of leukemia during 3 months observation and none resisted subsequent challenge with leukemic material.

Discussion. It is, of course, generally recognized that certain samples of normal human or animal sera may be toxic for certain cells in tissue culture(4,5). On the other hand, cytopathogenic activity, which is serially transmissible in tissue culture passages, has been observed in controlled experiments in which leukemic material was introduced into suitable tissue culture media. Such effects have been reported by Dmochowski *et al.* (6) with monkey kidney cell cultures inoculated with cell-free extracts from human leukemic lymph nodes and by De Carvalho(7) who inoculated primary human amnion cell

cultures with RNA extracted from human leukemic tissues. Further efforts by these authors to identify these cytopathogenic agents by injection of mice with tissue culture material led to inconclusive results with one investigator(6) whereas the other(7) recorded a significant incidence of leukemia in the injected animals. It is against this background of experimental experience by others that our own results appear to be of interest. Judging from our data, there seems to be no question that leukemic guinea pig plasma or spleen extract, both of which are capable of transmitting the disease in susceptible guinea pigs, contain quite frequently a factor which destroys normal cells of the same species in tissue culture. Yet, our work offers no evidence to show that this factor is self-replicating *in vitro* or will induce transformation of normal host cells into malignant cells in tissue culture. The nature of the cytotoxic factor, therefore, remains unknown. It is possible that it represents a nonspecific by-product of the disease, due to some metabolic disturbance, which accumulates in blood and tissues at the height of the leukemic process.

Summary and conclusions. A cytotoxic effect is described which occurs when leukemic guinea pig plasma is added to normal guinea pig spleen cells growing in primary tissue culture. The nature of the cytotoxic factor is unknown.

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Weight of Adrenal Glands of Cats. (25946)

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Jones(1) states that "A vast amount of information has been collected about organ weights of the laboratory rat, a fair amount for man, the guinea pig and dog, some for the rabbit, cat, mouse and hamster . . ." Since knowledge of the weights of endocrine glands is often of great help in estimating functional status, and data on weights of adrenals of the adult domestic cat are none too plentiful, we are here reporting on a larger series of cats than has heretofore been published. Only 5 papers were found in the literature giving data on weight of cat adrenals, and in only 2 of these is there anything like an adequate series. Latimer(2) reports on 52 males and 52 females, with adrenal weight-body weight ratios of 0.140 and 0.150 g/kg respectively. Butterworth and Mann(3), in connection with another study, give data on 41 males and 48 females, with ratios of 0.131 and 0.148. These authors also report a ratio of 0.111 for 21 castrated males. The other papers have series of 10 or less. Elliott and Tuckett(4) reported on 5 males and 9 females, with ratios of 0.118 and 0.153. Hill(5) gives a ratio of 0.125, based on adrenals of 3 females. Crile and Quiring(6) give a combined ratio of 0.337 for 6 males, 3 females, and one cat of unidentified sex. Surprisingly, the Handbook of Biological Data(7) ignores all previous data and reports a combined average adrenal weight-body weight ratio of 0.2 from 7 males and 3 females.

Methods. The material consists of 85 male