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Immunization of Mice Naturally Susceptible to a Transplantable Leukemia.

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The transplantable leukemia designated as line I[1] was started from a spontaneous case of lymphatic leukemia in April, 1929. The line has passed through 441 transfer generations and, by routine technique, has been inoculated in massive doses into 3625 mice of the highly inbred strain C 58; all but one of these died with the leukemic indications characteristic of this particular line of cells. The single survivor, which at no time showed clinical effects of the inoculation, was inoculated in the 77th transfer generation, in December, 1930. In the 3¾ years since that time, the 2925 mice from strain C 58 that have been inoculated with the massive standard dose of cells of line I have all developed leukemia. In the light of this record the natural susceptibility of strain C 58 to leukemic cells of line I appears to be fully established. The present experiments, started in April, 1934, are based on 100% susceptibility to the standard dose.

It has been shown previously[2] that between certain limits, reduction of the dosage lengthens the interval before death, and in the early transfers of line I that were used for these experiments (transfers 27-34, Jan.-Mar., 1930) the 18 mice given doses of 6000-9000 cells did not die with leukemia. However, these mice were not tested for an immunizing effect of surviving the small doses.

In returning to the study of small doses of the leukemic cells of line I, 346 transfer generations later, the virulence of the cells had become considerably enhanced and it now is found that the minimum dose that will kill is reduced to the order of magnitude of 200 cells. In successive dilutions of the massive standard dose the interval before death is progressively lengthened, as previously reported,[2] but a dilution is reached (1/1024th of standard) that permits a few of the mice to survive and as the dose is further reduced the proportion of survivors increases until every mouse survives (1/524,000th of standard). The percentage variation in cell number of different doses of a highly dilute suspension is probably

[1] Richter, M. N., and MacDowell, E. C., *J. Exp. Med.*, 1930, **52**, 823; Potter, J. S., and Richter, M. N., *Proc. Nat. Acad. Sc.*, 1932, **18**, 298.

[2] Richter, M. N., and MacDowell, E. C., *J. Exp. Med.*, 1933, **57**, 1.

very great, yet the consistency of the biological results from different dilutions bespeaks a fair approximation of the actual to the theoretical cell content.

Survivors of small doses were found to be resistant to progressively larger doses until the standard dose (in the order of 80 million cells) was given without harm. In some cases considerable growth of the cells inoculated in the first and second treatment was indicated by large spleens and general sickness followed by recovery, but in most cases there was no clinical evidence of any growth of the inoculated cells.

The results of inoculation with various dilutions and repeated reinoculation of survivors are given in Table I. Numbers in the

TABLE I.

Dilute Doses of Leukemic Cells (Line I) in Mice of Strain C58.
S = Standard dose; dilutions of standard dose indicated by negative exponents of 4; 2-3 weeks between successive doses.

Standard dose not given			Standard dose given		
Treatment	Death	Survival	Treatment	Death	Survival
—1	4	0	S (3¾ years)	2925	0
—2	4	0	—5, S	0	8
—2.5	4	0	—7, S	2	13
—3	25	0	—7, —3, S	0	1
—4	24	0	—7, —5, S	0	2
—5	24	8	—9, —5, S	1	4
—6	25	7	—6, —5, —3, S	0	1
—7	25	37	—7, —5, —3, S	0	12
—8	2	2	—9, —7, —3, S	0	6
—9	9	83	—9, —7, —5, S	1	9
—9.5	0	48	—10, —7, —5, S	0	4
—10	0	8	—6, —7, —5, —3, S	0	2
—11	0	4	—7, —7, —5, —3, S	0	2
			—7, —9, —7, —5, S	0	1
—9, —5	3	5	—8, —9, —7, —5, S	0	2
—9, —7	3	46	—9, —7, —5, —3, S	0	16
—9, —9	2	24	—9, —9, —7, —5, S	0	2
—10, —7	1	7	—10, —7, —5, —3, S	0	3
			—11, —7, —5, —3, S	0	4
			—9, —9, —7, —5, —3, S	0	22
				Total	114

columns headed *treatment* indicate dilutions of the standard dose (S); these numbers are negative exponents of 4, so that —1 indicates a dilution of ¼th and —11, 1/4,194,304th of the standard dose. The interval between successive inoculations was usually 2-3 weeks. Mice surviving doses less than standard (entered in the left hand side of the table) in most cases reappear in the right hand side. Animals listed as survivors of the standard dose have shown no sign of leukemia for at least 16 days; this is 4 times the interval

between inoculation and death of the controls. At present 58 survivors of the standard dose have been under observation for at least 3 months.

Although many variations in procedure prevent any simple statement of the degree of success obtained, the phenomenon of active immunization of mice naturally susceptible to massive doses of a certain line of leukemic cells appears to be established by the 114 mice that have been immunized to resist the standard dose, in contrast to the 2925 non-immunized mice of the same strain that have been inoculated since 1930 with the same standard dose without a single survivor.

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Vaccination of Rabbits Against Intradermal Pneumococcus Infection.

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A vaccine of Type I pneumococcus was prepared by cultivating a highly virulent strain in broth for 24 hours at 37°C. The culture was thoroughly centrifuged, the supernatant broth discarded and the pneumococci suspended in sterile distilled water to give approximately 1000 million per cc. This suspension was heated at 60°C. for one hour, cultured for sterility and preserved with 0.3% tricresol.

Similar vaccines were prepared of Type II and Type III pneumococci.

Results with Type I Vaccine. Six adult rabbits were given 1 cc. of Type I pneumococcus vaccine per kilo of weight by subcutaneous injection every 5 days for 5 doses. One week after the last dose all, along with 2 controls, were inoculated intradermally with 0.2 cc. of 18-hour broth culture after the method of Goodner.[1] The controls developed the typical local lesions, associated septicemia, fever, leukocytic changes, etc., and succumbed 4 to 5 days after the inoculation.

All of the 6 vaccinated rabbits survived. Local lesions of slight to moderate severity developed in all with positive cultures of the edema fluid over 1 to 2 days following inoculation which were thereafter sterile. Daily blood cultures were negative in 5 animals;

[1] Goodner, K., *J. Exp. Med.*, 1928, **48**, 1, 413; 1931, **54**, 847.