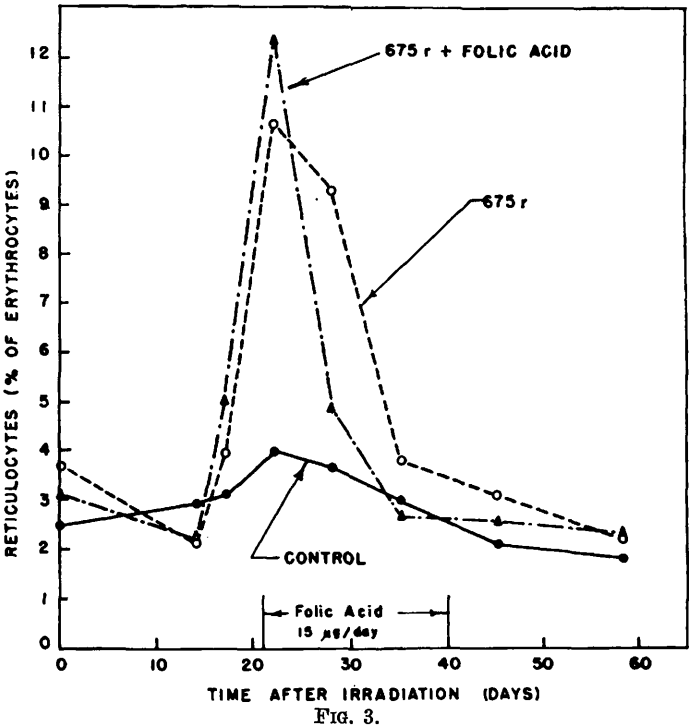

FIG. 3.
Effect of 675 r X irradiation and administration of folic acid on % of reticulocytes in the peripheral blood.

bone marrow is probably caused principally by direct injury. Any indirect damage resulting from damage to the viscera, which produces the antianemia principle, must be a relatively minor source of injury.

16776

Genetic Changes in Gastric Lesion and Fibrosarcoma Susceptibilities.*

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Evidence has been published that the gene for brown hair has mutated to dominant black several times in the descendants of mice which had been injected with methylcholanthrene for several generations. Concomitant with the production of these dominant hair color mutations there were also obtained (1) a considerable increase in susceptibility to induced fibrosarcomas, (2) an increased litter size, and (3) increased vitality. All 3 of these characteristics appear to be associated with the black mutant character, never with their brown litter mates. The evidence points toward the production of a widespread germinal change brought about by the effect of methylcholanthrene upon the germ plasm,

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but a point mutation at a single locus has not as yet been completely excluded.[1]

It has also been demonstrated that the gene for susceptibility to induced fibrosarcomas in mice shows linkage relationship with the gene for black hair pigmentation. The essential data of crossing over between these two genes on the brown tagged chromosome have already been obtained.[2]

A gene for susceptibility to a stomach lesion occurring primarily in the gastric mucosa of NHO mice also shows linkage relationship with the brown gene.[3]

The present paper reports the data obtained on the incidence of gastric lesions following the subcutaneous injection of methylcholanthrene in the black mutants and their litter mate brown segregates. Of the black mutants 6 descents have been continued for several generations, 3 of these descents were obtained from a brother-sister mating of two of the original black mutants, whereas the other 3 descents were descended from a backcross between one of the black mutants and its own mother of the original brown ancestry. Each of these 3 descents were made up of (a) pure breeding blacks, (b) derived pure breeding brown segregates, and (c) black heterozygous for brown. All mice were weaned at 30 days of age, mated together and injected subcutaneously in the right groin with 1 mg of methylcholanthrene dissolved in 0.1 cc of sesame oil at 60 days of age. They were examined periodically for the appearance of tumors and signs of sickness. All mice were killed when progressively growing tumors were evident or when they showed one or more signs of sickness, such as emaciation, wheezing or a ruffled appearance of the hair.

It is impossible to demonstrate whether these 3 black mutants which appeared in one litter of mice were derived from one original mutation or from 3 separate ones. If the mutation occurred in one germinal epithelial cell and then the mutant cell gave rise to 3 eggs, it is possible that one mutation was responsible for all 3 mutants. This interpretation is consistent with the embryology of the mouse. There are further considerations that only one mutation was involved. (1) It is highly improbable that any inductive system no matter how specific in action would produce three mutations at the same locus in 3 separate animals without inducing other mutations at other loci. This did not occur. (2) The 3 mutants are the same as far as the somatic manifestation of the mutant gene is concerned. They are each characterized by a peculiar distribution of pigment granules in the hairs which simulates but differs somewhat from the pigment distribution in the well known black mutation, as found in mice of the C_{57} black stock.[3] All 3 black mutants gave comparable Mendelian ratios when crossed with each other or in the F_2 and backcross generations to the recessive condition, brown, following an outcross to mice of the Strong A strain. For these reasons it is considered that the three mutants are genetically and biologically similar if not identical and the data obtained on the descendants of all these can be legitimately classified together.

Of these mice derived from the black mutants and injected with methylcholanthrene, 362 developed lesions involving the stomach, as follows: 198 black to 164 browns. These gastric lesions consisted of several histological types, comparable to the varieties already obtained with methylcholanthrene in the original brown ancestry. There were 3 distinct regions of the stomach of the mouse which gave rise to neoplasia, as follows: (1) a squamous type from the fore-stomach, (2) mixed types from the region of the limiting ridge, and (3) the lesions just anterior to the pylorus involving the mucus secreting neck cells of the gastric mucosa. In the black series the sex differential of gastric lesions was 1.95 males to 1.00 females, whereas in the brown series the sex differential was 2.40 males to 1.00 females. The distributions of gastric lesions for the two color classes of mice are given in Chart 1: brown mice on the short dash line, black mice on the solid line.

[1] Strong, L. C., *Yale J. Biol. and Med.*, 1946, **18**, 359.

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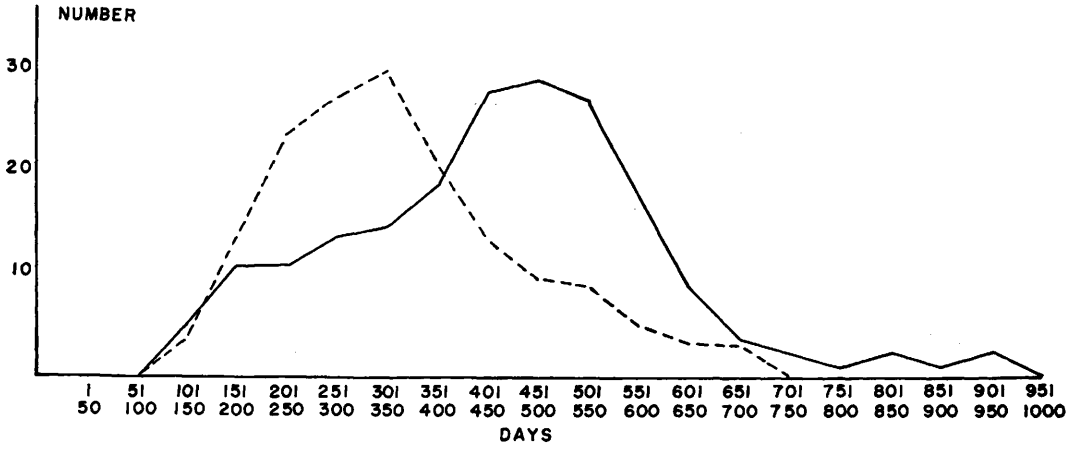

CHART 1.

This chart presents the data on the frequency distribution of induced gastric lesions in mice of the Bl subline; time in days is given on the base line, the number of mice on the vertical line. Black mutants are given on the solid line, brown segregates on the short dash line.

The average latent period for the appearance of the gastric lesions in black mice was 434.4 days, whereas the average latent period for the brown mice was 335.8 days. This is a difference between the two color classes of 98.6 days. The average latent period of gastric lesions in the original brown descent from which the black mutation had occurred was 318.5 days. Thus it is evident that the brown segregates from the black mutants retain the same latent period for gastric lesions as their brown ancestors (335.8 days-318.5 days = 17.3 days difference) whereas the black mutants have acquired a delayed latent period of 115.9 days beyond the original brown ancestry (434.4 days - 318.5 days = 115.9 days).

The present data on the incidence of tumors induced in black mutants by the subcutaneous injection of methylcholanthrene has therefore, led to the conclusion that while one type of tumor susceptibility (fibrosarcomas at the site of injection) is being increased, another type, the gastric lesion, is being decreased. This is evidence that in this particular experimental setup the two cancer susceptibilities are distinct entities even though they both show linkage relationships with the brown tagged chromosome. The exact spatial relationship of the 3 entities or genes on the brown chromosome cannot at present be determined. Another conclusion that seems justified is that in the origin of the original black mutant more than a single point mutation was involved. This finding is of increased significance, since, as far as can be determined now, the original mutation from brown to black itself was a clear cut point mutation, as no variations from typical Mendelian ratios could be determined in the inheritance of mutant black. Skewness to the right of the frequency distribution curve for gastric lesions in brown mice and to the left for the black mutants is suggestive that this may be brought about, in part, by the two cross-over types, a brown mouse with a delayed latent period for a gastric lesion and a black mouse with an enhanced or original early appearance of the gastric lesion. Unfortunately no progeny from these mice were obtained so that this genetic evidence of crossing over could not be obtained.

The present evidence also indicates that

TABLE I.

	Bl descent		
	Black	Brown	Total
Gastric lesions	198	164	362
Normal	2145	1452	3597
Total	2343	1616	3959

This table presents data on mice of the Bl descent injected with methylcholanthrene at 60 days of age. The mice are classified according to whether they developed gastric lesions or did not develop such a lesion. The mice are further divided into the two color classes, as follows (a) the original color class brown and (b) the mutant black.

there are specific entities or genes underlying genetic susceptibilities to specific types of neoplasia induced by methylcholanthrene. This interpretation seems to be favored over the alternative concept that in cancer susceptibility, there is a general cancer "gene" that underlies all types of cancer. That is, that there is a gene that determines the difference between the biological states of cancer and not cancer.

16777

Mucolytic Enzyme Systems. IV. Relationship of Hyaluronidase Inhibition by Blood Serum to Incidence of Mammary Cancer in Mice.*

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From previous investigations in this series, it was concluded that the level of hyaluronidase inhibitor in blood serum is elevated in a wide variety of both virus and bacterial infections[1-3] as well as in malignant processes.[4] While these investigations were still in progress Friou and Wenner[5] reported a similar elevation in rheumatic fever as determined by the mucin clot test, and Thompson and Moses[6] also found this effect in pneumonia by the clot test. Fulton, Marcus and Robinson[7] used a method based on decapsulation of a group A hemolytic streptococcus to measure the inhibition by serum and reported no significant differences between normals, and patients with rheumatoid arthritis.

The inhibiting factor which undergoes change is distinct from the specific antibody inhibitors which are elicited in response to hyaluronidase acting as an antigen. By electrophoresis at pH 8.6 it was found that the non-antibody inhibitor in the serum migrated chiefly with the albumin.[8] The antibody inhibitor would be expected to be found in the gamma globulin fraction. The factor in question appears to be a non-specific inhibitor since it is capable of inhibiting the enzyme from diverse sources, and furthermore its elevation seems to be a non-specific response to both infection and malignancy. While it is still premature to advance a theory, one might postulate that this response is a defense mechanism designed to counteract the invasiveness potentiated by hyaluronidase. While many organisms do not in themselves possess hyaluronidase, their invasiveness appears to be enhanced, nevertheless, by the presence of the enzyme,[9] and, accordingly, it is conceivable that a general response against hyaluronidase activity might be employed as a general defense mechanism.

The possibility that hyaluronidase may be involved in the invasive processes of cancer,[10-16] and the great elevation of the serum

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