of subsequent surgical intervention. For example, previous work which has implicated the caudate nucleus with deficit on delayed alternation in the monkey (*8*) could be supplemented by this approach. Since the extensive extirpation permits relatively easy access to some remaining subcortical structures, which are generally difficult to reach, it is believed that this technique can provide a useful tool for further study of the role played by the subcortex in various tasks (*9*).

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High Incidences of Transmissible Kidney Tumors in Uninoculated Frogs Maintained in a Laboratory

Abstract. Various workers have shown that the Lucké carcinoma, a transmissible kidney tumor of the leopard frog, occurs spontaneously at low incidence (6.7 percent maximum). However, we have consistently observed much higher incidences (50 percent maximum), a finding of probable significance in the epidemiology and natural transmission of the tumor. Age, metabolic level, and cross infection are being investigated as possible factors in determining the tumor incidence.

Kidney tumors of the type described by Lucké (*1*) are occasionally seen in leopard frogs (*Rana pipiens*) collected in the region surrounding Lake Champlain, Vermont. In the only extensive studies of spontaneous incidence, Lucké found tumors in 2.7 percent of newly caught frogs, and in 6.7 percent of uninoculated frogs kept in the laboratory for periods ranging from 6 months to 1 year or more (*2*). However, nine uninoculated groups maintained intermittently by us as controls in tumor transmission studies have in nearly every instance shown much higher incidences of tumors at the end of 6 months (*3*).

All groups are represented in Table 1, which shows that incidences ranging between 20 and 50 percent were observed between the 30th and 41st weeks. One particular group (group 5) was examined in greater detail. Of 87 frogs which survived for 30 weeks or died before that time with kidney tumor, 12 developed palpable tumors. The remaining 75, killed and autopsied at the end of 30 weeks, yielded two small tumors, as confirmed in histological sections, and four cases in which blanched, nodular areas were seen in the kidneys. On section, three were seen to be helminthic in origin, but the fourth was a small, although a typical, case of Lucké tumor.

Finally, normal-appearing kidneys from ten male and ten female frogs were fixed, and center segments representing one-fourth of each pair were sectioned serially. This method was adopted in order to survey the material without the laborious process of sectioning entire kidneys. The conservative assumption was adopted that histologically normal center sections represented normal kidneys. Among the 20 pairs of kidneys so sectioned, two were found which bore microscopic but typical and unmistakable tumor foci. Thus, 17 tumors (20 percent) were found among the original 87 frogs. However, it is estimated, on the basis of the two microscopic tumors found, that five microscopic tumors might have been expected in the remaining 52 pairs of kidneys which were grossly normal in appearance, but which were not sectioned. If so, the total number of tumors present in the group would be 22 or 25 percent.

Table 1 indicates that a considerable increase in the number of tumors is to be expected after 30 weeks. For that reason, the 18 remaining normal-appearing pairs of group 5 kidneys which were sectioned were also scored for the presence of the presumptive tumor transitional changes observed by Duryee (*4, 5*). Such changes were described by him on the basis of abnormal cells which occurred in tumor-bearing kidneys and in kidneys of frogs inoculated with Lucké tumor filtrates.

Of the 20 pairs of kidneys which we scored, four showed advanced tubule changes suggesting borderline neoplasia, while six others showed slight-to-moderate change of the same character. These ten cases consisted of occasional nephrons in which the proximal convoluted portions of the tubules showed nucleolar enlargement, pronounced basophilia, numerous cytoplasmic inclusions of various types, and mitotic figures (see *4*). Similar changes were not seen by us in the kidneys of several dozen frogs killed immediately upon arrival in the laboratory, but the changes were abundantly seen in a majority of cases in which frogs were inoculated with tumor-derived materials and examined histologically at a later date.

On the basis of an assumption, currently being tested, that kidneys bearing nephrons of transitional appearance are destined for neoplasia, it is estimated that of the total group of 87 frogs, 41 percent (36 individuals) either bore kidney tumors (25 percent) or were in preneoplastic condition (16 percent), if only the four advanced cases of transitional change are considered. As shown in Table 1, this figure agrees well with the incidence actually found in groups which were maintained a few weeks longer before their death, and suggests that such kidneys were destined to become grossly neoplastic within about 8 weeks.

When all ten cases with nephrons of the presumed transitional type are considered, it is estimated that 66 percent of the total group (25 percent bearing kidney tumors, plus an additional 41 percent in the transitional state) bore the tumor or were destined to develop tumors. This figure should be compared with Duryee's figure of 56 percent for combined transitions and kidney tumors in frogs inoculated with tumor filtrates and examined after 4 months or less (*4*).

The factors responsible for the observed high incidence of Lucké tumors are not yet known. However, environmental factors related to temperature and metabolic level, to age, and possibly to opportunities for laboratory cross-infection or contamination differed

Table 1. Occurrence of kidney tumors in uninoculated frogs maintained in the laboratory.

Group	Frogs (No.)	Time kept (wk)	Kidney tumors	
			(No.)	(%)
1	10	9	1	10
2	20	11	1	5
3	12	13	3	25
4	20	24	0	0
5	87	30	17	20
6	8	31	2	25
7	10	33	5	50
8	10	39	5	50
9	20	41	7	35
Total	197		41	
Mean		30		21

in these experiments from those reported by others who have not observed high incidences of the tumor (*2, 4*). Experiments are planned to determine which, if any, of the factors named can influence the tumor incidence.

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4 November 1960

Interaction of Chromatid Breaks Produced by X-rays and Radiomimetic Compounds

Abstract. The results of combination treatments of the roots of *Vicia faba* with certain radiomimetic compounds (8-ethoxycaffeine, maleic hydrazide, β-propiolactone, potassium cyanide) and x-ray as well as combination treatments of certain radiomimetic compounds with one another were observed to determine whether interaction will occur between chromosomal breaks induced by different agents. Interaction was observed between breaks induced by x-rays and all of the breaks induced by chemicals but not between breaks induced by any two chemicals. The results are discussed in terms of possible breakage bond differences and the effects of temporal and spatial differences in breaks induced by different agents.

It has been known for some time that treatment with certain chemicals classed as radiomimetic results in chromosomal aberrations that are indistinguishable, when observed at metaphase, from radiation-induced changes. Whether or not the breaks induced by these compounds are qualitatively similar to one another and to radiation-induced breaks is still a moot point. Evidence at this time indicates that regardless of the similarity or dissimilarity of the breaks, the areas in which the breaks induced by a given compound occur are often quite specific. For example, in *Vicia faba*, the chromosomal breaks induced by 8-ethoxycaffeine are most frequently located in the nucleolar organizer region of the satellited chromosomes. Breaks induced by maleic hydrazide are most frequently observed in the two segments of heterochromatin located on either side of the centromere of the satellited chromosomes. Breaks induced by radiation seem to be more at random. They are observed in euchromatic as well as heterochromatic regions, and the frequency with which breaks occur in the long as against the short chromosomes approaches randomness.

Wolff and Luippold (*1*), in their studies of the rejoining system, and Cohn (*2*), in his study of the interaction of x-ray-induced breaks, have demonstrated that whatever the nature of the bonds broken during treatment with x-rays there is sufficient similarity between enough of these ruptured bonds to result in the interaction of broken ends. That all or most x-ray-induced breaks are similar enough to interact is indicated by the fact that Cohn observed a frequency of interaction as great as that which he calculated for complete interaction (*2*). It is feasible, in the light of existing evidence, to consider that all breaks, no matter what their origin or chemical nature, interact through the mediation of a common repair system that does not distinguish between one broken bond and another so that the difference between an interchange and a noninterchange situation is a function only of availability of the breaks in terms of time and space and not of chemical differences between the bonds that are broken.

It is of some interest to test the hypothesis that all breaks, no matter what their nature, can interact with one another. One method would be to confront the "rejoining system" with breaks induced simultaneously by different agents and observe the amount of interaction taking place. These breaks would be randomly dispersed or concentrated in specific localities in the chromosomes, depending on the agent or agents employed.

Specificity in terms of localized breakage can be explained in two ways. First, the compound may be incorporated at specific sites and is therefore restricted in its action to a few localities. The only evidence bearing on this point makes this an unlikely hypothesis since autoradiographic studies of tritiated 8-ethoxycaffeine indicate that at least this compound is not incorporated at the site where most of the damage is observed (*3*). Second, specificity can be explained by assuming that an agent inducing breaks in specific localities in few or all chromosomes is capable of producing breaks in one or few kinds of bonds which occur rarely along the length of the chromosomes. Conversely, an agent inducing breaks which are randomly located along the length of all chromosomes can be considered capable of producing breaks in several kinds of bonds, or, assuming some specificity of action, of breaking a particular bond that is exceedingly common along the length of the chromosomes. Regardless of the explanations for observed localized breakage, it is of interest to test whether or not breaks induced at specific sites by particular agents interact with other breaks, randomly or specifically located.

The experimental material used in the following experiments (*4*) consisted of lateral root tips of the broad bean, *Vicia faba*. The variety used, Seville Long Pod, was obtained from Carter's Tested Seeds Limited, London, England. The roots were grown in shell vials in the dark at 25°C for 6 days following an initial 24-hour period of soaking. The tap water was changed twice daily during growth and recovery (the latter being the time interval between treatment and fixation). Twenty-four hours before treatment the roots were placed in an incubator at 17°C. Treatments were performed at 20°C, and recovery took place at 17°C, in the dark. The root tips were treated with 0.05 percent colchicine for 3 to 4 hours before fixation in a cold mixture of alcohol and acetic acid (3:1), and slides were prepared as Feulgen squashes. The treatments were carried out in shell vials and were performed in three different ways.

The two agents that induce breaks whose ability to interact was being tested were introduced simultaneously, and each was used as pretreatment for the other. The agents used to induce specific and randomized breaks are as follows: β-propiolactone, potassium cyanide, 8-ethoxycaffeine, maleic hydrazide, and x-rays. Dinitrophenol was also used in an occasional experiment to keep the breaks open between treatments (*1*) to determine whether or not it had any effect on the exchange frequency (interaction) (*2*). The following molar concentrations of the various agents were use: β-propiolactone, 7×10^{-3}; KCN, 1×10^{-3}; 8-ethoxycaffeine, 1×10^{-2}; maleic hydrazide, 2×10^{-4}; dinitrophenol, 1×10^{-4}. The x-ray dose, when used, was 100 r.

Results of the different combination treatments are summarized in Table 1. The experiments listed here are based on the assumption that x-ray-induced breaks, being more or less randomly distributed, are likely to include amongst them enough broken bonds similar to those induced by radiomimetic compounds that interaction will result from combination treatments including radiation as one of the agents. Conversely, combination treatments with two agents that induce breakage in relatively localized areas are likely to break few, if any, similar bonds, and therefore