cence was found only in non-necrotic areas of either primary or metastatic tumors, although it also occurred in areas of edema or cyst formation. In experiments made to determine whether this report could serve as the basis for a method of determining viable areas of mouse tumors prior to transplantation, and thus of reducing the incidence of failure of take, the following results were obtained.

TABLE 1

DISTRIBUTION OF FLUORESCEIN IN SARCOMA 180 AT INTERVALS AFTER INTRAVENOUS ADMINISTRATION

Time	Fluorescence	
	Animal tissues*	Tumors†
Under 12 min	Marked	Nonuniform fluorescence, tending to be higher on periphery
12 min–3 hrs	Marked early to none at 3 hrs, with some variability	Uniform marked fluorescence
3–7 hrs	None	Marked fluorescence in necrotic areas: tended to decrease by 7 hrs

* Fluorescence was present at all times in the colon, gallbladder, and urinary bladder—the normal routes of excretion of fluorescein (*1*).

† In a few animals with spontaneous mammary carcinoma, this tumor showed essentially the same sequence of changes in distribution of fluorescein as the transplanted sarcoma 180.

Carworth Farm mice weighing 20–25 gm and bearing 15-day-old transplants of sarcoma 180 (with some variation in size) were injected intravenously with 0.25 cc of a 1:14 dilution of saturated fluorescein in normal saline and killed at various times after injection by crushing the cervical cord. The animals were opened, the tumors hemisected, and both animals and tumors examined under ultraviolet light. Sixty-eight mice (136 tumors) were studied in groups at intervals from less than 1 min after injection up to ½ hr, and at ½-hr intervals to 7 hrs. The findings in the various groups are summarized in Table 1.

FIG. 1. (a) Section of tumor at 5 mμ; pale central area is necrotic. (b) Diagram of same tumor at autopsy; outlined central area showed fluorescence. Note that the necrotic and fluorescing areas correspond.

Microscopic sections of all tumors showed the presence of irregular areas of necrosis. Fig. 1b shows the area of fluorescence observed in one tumor; Fig. 1a, the section made from this tumor to show the essential identity of the area of fluorescence and the area of necrosis.

It is apparent from these data that the distribution of fluorescein in sarcoma 180 after a constant intravenous dose is determined by the length of time between injection and observation and by the amount of necrosis; at the longer durations the fluorescence was thus observed predominantly or only in the areas of necrosis. These results suggest that, in these tumors at least, a major factor determining the distribution of fluorescein in the tumors at any given time after injection is the slowness of diffusion into and out of poorly vascularized or necrotic areas.

Relative staining of viable and nonviable portions of tumors with fluorescein must thus be considered a dose-duration phenomenon, rather than the result of any specific staining potentiality of either portion.

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Physiological Adjustments in Chloride Balance of the Goldfish

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Absorption of certain ions, particularly chloride and sodium against an osmotic gradient, by the gills of certain fresh-water fishes was demonstrated by Krogh (*1*). The goldfish proved to be a favorable subject for these experiments, but in the case of chlorides, at least, Krogh (*2*) states that the body must be depleted below normal ion concentration of the internal medium before absorption occurs. He accomplished this by leaching the chlorides from the fish with distilled water. However, there are no quantitative studies showing that an initial reduction of the chloride level is necessary to evoke absorption of this ion. In the present studies data were obtained upon this phase of the chloride problem.

Vigorous goldfish (body weight, 45–95 gm) from a commercial hatchery which had been kept for at least 5 days without food in tanks of flowing water were used. Throughout the experiments each fish was held in a glass battery jar containing a carefully measured volume of tap water aerated continuously with washed, compressed air. In those situations in which it was necessary to separate the activity of the gills from that of the kidneys, a thin rubber sack was slipped over the tail of the fish and brought forward to the level of the pectoral fins, where it was bound snugly to the fish with cotton thread. Preliminary experiments had shown that this procedure did not interfere with equilibrium or locomotion of the fish. The urine and feces formed were collected in the rubber sack, but since the fish were in a fasting state, the material from the alimentary tract was negligible. The movements of the chloride ions were determined by analyses of the water surrounding the fish

and of the urine collected in the rubber sack. The electrometric titration of chlorides was followed, employing the electrode system of Yeck and Kissin (*4*).

Using a series of 80 goldfish, 164 determinations of the chlorides in the water surrounding the individual fish over periods ranging from 20 min to 117 hrs were made. The data have been summarized in Table 1. These fish

TABLE 1

LOSS OF CHLORIDES FROM AND ABSORPTION BY THE GILLS OF GOLDFISH

Duration of experiment	No. of determinations	Mg of chloride/gm of body weight lost (−) or absorbed (+) by the fish	
		Range	Mean
20–60 min	11	0.000 to −0.035	−0.019
4– 6 hrs	27	0.000 to −0.097	−0.033
23–24 "	39	−0.027 to +0.296	+0.144
44–52 "	78	+0.105 to +0.928	+0.375
72 "	3	+0.647 to +0.920	+0.805
96 "	3	+0.650 to +1.010	+0.850
117 "	3	+0.600 to +0.935	+0.798

were taken directly from the concrete holding tank, the rubber sack secured around the posterior portion of the body, and the individual fish placed in the experimental jar without further treatment.

Within the first 20 min there was an appreciable loss of chloride ion from the fish into the surrounding water. This loss continued at a diminishing rate for 4–6 hrs, at which time the absorption of chlorides was begun. With few exceptions, all the chloride lost by the fish into the water had been regained and additional chloride absorbed by the end of 24 hrs. In the period between 24 and 48 hrs the chloride concentration of the water surrounding the fish was markedly reduced. In several cases as much as 34 mg of chloride was absorbed by individual fish, and the concentration of that ion reduced in the water to 0.006 mM/liter. In most cases, by the end of 48 hrs the chloride content of the water was reduced so low that the experiment was terminated. However, in three cases, sodium chloride was added to the water and the absorptive process followed for 117 hrs. The chloride uptake by the fish continued through 72 hrs, leveling off at 96 hrs and dropping slightly at 117 hrs.

In this series it is evident that both the initial loss of chloride by the fish and the subsequent absorption of chloride from the surrounding water were accomplished via the gills, as all of the urine and any material voided from the alimentary canal were caught in the rubber sack. In view of other experimental work it is highly improbable that any chlorides were absorbed from the rubber sack through the unbroken and uninjured integument of the fish.

Another lot of 36 fish was divided into three groups, each fish of one group receiving by intraperitoneal injection 2.5 ml of glass-distilled water and each fish of the second group, 2.5 ml of a sodium chloride solution carrying 50 mg of the chloride ion. The third group consisted of uninjected controls.

The chloride determinations on both the surrounding water and the urine in the rubber sacks were made at the end of 48 hrs. The data are given in Table 2.

TABLE 2

EFFECTS OF INJECTIONS OF CHLORIDE OR DISTILLED WATER UPON THE CHLORIDE ABSORPTION (GILLS) AND EXCRETION (KIDNEYS) OF THE GOLDFISH AT THE END OF A 48-HR PERIOD

Groups	No. of fish	Mg of chloride/gm of fish absorbed or excreted	
		Range	Mean
Absorption			
A Controls	11	0.168 to 0.480	0.315
B Salt injection	11	0.055 to 0.433	0.189
C Distilled-water injection	14	0.184 to 0.390	0.301
Excretion			
A Controls	11	0.271 to 0.470	0.368
B Salt injection	12	0.230 to 0.550	0.361
C Distilled-water injection	13	0.160 to 0.398	0.258

Statistical significance—Absorption: A : B, $t = 2.595$, $P < 0.02$; A : C, $t = 0.407$, $P < 0.7$; B : C, $t = 2.92$, $P < 0.01$. Excretion: A :B, $t = 0.197$, $P < 0.9$; A : C, $t = 4.141$, $P < 0.001$; B : C, $t = 2.915$, $P < 0.01$. A P-value of less than 0.02 is considered to be statistically significant.

There was a statistically significant difference in absorption between the fish receiving 50 mg of the chloride ion and those receiving distilled water, the average amount absorbed being 0.189 mg/gm of body weight for the former as compared with 0.301 mg/gm for the latter group. There was no significant difference between the absorption of chloride by the uninjected controls and the distilled-water group. On the other hand, excretion of chloride ion was reduced in the fish receiving the distilled-water injection, the average being 0.258 mg/gm as compared with 0.368 mg/gm for the salt-injected fish and 0.361 mg/gm for the uninjected controls. This difference was also statistically significant.

It is apparent that excitation of the fish by handling is sufficient to initiate changes in the absorptive mechanism, the fish first losing chlorides from the gills, before the absorption began. Krogh (*1*) states that goldfish would not absorb chlorides immediately when placed in an experimental chamber, but his "washing out" technique obscured the fact that a preliminary loss of this ion occurred as a result of excitement. These reactions of the goldfish are suggestive of the adaptation syndrome of Selye (*3*).

The initial loss of chlorides from the gills during the first 4 hrs of these experiments amounted to about 3% of the entire quantity of that ion present in the body of

the fish. This loss, if there were no compensation from the tissues, would lower the blood chlorides only from ca. 440 mg% to 427 mg%—relatively a very small change, for both of these values lie within the range of normal blood chlorides for the goldfish. It is uncertain whether this constitutes sufficient depletion to activate the chloride-absorbing mechanism, but it is evident that once the absorption was begun, reducing the chloride concentration of the body (by dilution) tends to retard excretion of the ion rather than to accelerate absorption. Conversely, increasing the chloride concentration of the body fluids (by injecting NaCl) retards absorption but does not increase the rate of excretion.

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Transmission by Leaf Hoppers of the Virus Causing Phloem Necrosis of American Elm

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Phloem necrosis, a virus disease, is one of the most destructive diseases affecting the American elm. Its origin is unknown, although observations and reports by Garman (*5*) and Forbes (*4*) indicate its presence in the Ohio River Valley as early as 1882. In recent years this disease has become epidemic in many sections and has destroyed thousands of valuable shade trees in such cities as Columbus and Dayton, Ohio; Peoria, Illinois; and St. Louis and Kansas City, Missouri. It has become widespread and is now known to occur in Ohio, Indiana, Illinois, Missouri, Iowa, Nebraska, Kansas, Oklahoma, Arkansas, Mississippi, Tennessee, Kentucky, and West Virginia (*1*).

In 1940 the Bureau of Entomology and Plant Quarantine, in cooperation with the Bureau of Plant Industry, Soils, and Agricultural Engineering, established a laboratory at Columbus, Ohio, for the purpose of studying the disease and determining the possible insect vectors of the virus. This paper reports briefly transmission studies with some of the insect species under investigation.

Late in August and early in September 1940, some adults of the leaf hopper genus *Erythroneura* were collected from elm in the Columbus area and confined for 4 days in cloth sleeves placed over the foliage of diseased elm trees. After this period of feeding the insects were divided into two lots and placed in two cloth-covered cages ($6' \times 6' \times 9'$), each of which contained 4 healthy elm seedlings of approximately $\frac{3}{4}''$ caliper. The insects were not disturbed again and were left to feed until they died. The cages containing the seedlings were kept covered during the active insect seasons through 1942. No disease symptoms having developed meanwhile, the cages were then removed and the trees left to grow unprotected thereafter. Each succeeding year the trees were examined for signs of disease. In August 1945, 3 of the 8 trees showed typical symptoms of phloem necrosis. Two of these died the same summer, and the third died early the following spring. The other 5 have remained healthy to date.

Further extensive tests with this group of leaf hoppers were established after the trees had developed symptoms of disease in 1945 and are now under way, but it will be some time before they will be completed. The results of the tests begun in 1940 are being reported, however, because they indicate that transmission was accomplished by insects in this genus. In addition to the three trees that died, only one tree among several thousand in the immediate vicinity has become diseased since 1940 without previous inoculation by diseased tissue grafting. The exception is a tree that grew in a cage adjoining the one in which two of the three died, the two cages having one cloth wall in common.

The *Erythroneura* specimens used in the tests begun in 1940 could not be recovered for identification, and the species involved remain unknown. More than one species probably was confined in each cage; yet the chances are that most of them were of the species *campora*, which is known to have been common in the collection area in 1940. This species occurs commonly on elm and doubtlessly is distributed throughout the disease region.

In early surveys of elm insects in the disease region several species of the leaf hopper genus *Scaphoideus* were collected. One of these, *S. luteolus* Van D., is a consistent inhabitant of elm. This species, although difficult to separate from closely allied ones in the adult stage, was found to differ markedly from others in the nymphal stages. This difference facilitated collection and permitted the establishment of a large series of transmission tests with this elm-inhabiting species from 1941 through 1943. The species was not used in tests in 1944, and only a few nymphs were used in 1945. In July 1946, however, a small number of nymphs were collected and, after they had fed for various periods on diseased elm foliage, were placed under test on healthy one-year-old American elm seedlings. On July 12, 2 seedlings were exposed to nymphs and adults that had fed the previous 9 days on diseased elm foliage; on July 15, 5 seedlings were exposed to nymphs that had fed the previous 12 days; and on July 26, 2 seedlings were exposed to nymphs and adults that had fed the previous 3 days. In all cases the infective insects were left on the test seedlings until the insects died. When all were dead, the seedlings were placed in a

[1] D. E. Parker supervised these investigations, H. T. Osborn conducted the laboratory tests prior to 1944, and R. U. Swingle, of the Bureau of Plant Industry, Soils, and Agricultural Engineering, gave valuable assistance in early diagnostic work.