have been obtained with mitochondria from liver.

As proposed in the drawing of Fig. 3, an increased rate of influx of labeled anions with acidification, or of cations with alkalinization, might be anticipated on the basis of change in permeability due to change in surface charge. Alteration in permeability would affect bidirectional flux and would serve to explain the changes with potassium, at least in the range between pH 7.2 and 8.5. On the other hand, if a net increase in content is involved, such as is the case with chloride below pH 7, permeability would be critical only if it were the rate limiting step. One may presume that cell membranes do have end groups with dissociation constants in the physiological range, and hence that the reaction pattern of Fig. 3 does have some importance generally. There is, however, little to go on with respect to quantitative significance. The results obtained with imidazole emphasize a synergistic effect due to change in charge of the free ions in solution.

Summary. Change in the pH of the incubation medium has been shown to have opposite effects upon influx rates of cations and of anions into mitochondrial substance. Alkalinization was associated with increase in influx of Na^+ and K^+; and conversely, acidification resulted in more rapid influx of Br^- and Cl^-. Increased sensitivity to pH change was observed in the presence of the buffer ion imidazole. At pH 7 imidazole carries a positive charge and inhibition to influx of Na^+ was observed. The inhibition was absent at pH 8.5 with conversion to the uncharged free base.

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Decreased Resistance to Tumor Cells after Stress, Followed by Increased Resistance.* (28370)

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Numerous workers have shown that various types of stress (celiotomy, liver damage, cold, etc.) will reduce the resistance of animals to inoculated cancer cells(1,2,4,5,6,7). Slawikowski(7) has shown that this reduced resistance lasts between 48 and 96 hours, and that the stress involved is different than the Selye stress. However, it is not known whether the animals develop an increased resistance to cancer cells after this period of reduced resistance terminates. Such a reversal or rebound effect is well known in many reactions involving immunity(8).

The reduced resistance to cancer after stress, referred to in most of the articles listed above, was demonstrated by an increased percentage take of cancer cells (Walker 256 carcinosarcoma) which were injected immediately before or after the stress. This increased take was noted in rats undergoing a stress, such as celiotomy or liver trauma, over the percentage take in control rats having an anesthetic, but no surgical stress. In general, the takes were about twice as high in those rats having the stress at time of cell injection as they were in the control rats not having stress at time of cell injection.

Materials and methods. In this experiment white female Sprague-Dawley rats (180-200 g) were used as the experimental animals. The stress induced was a celiotomy, performed under Nembutal anesthesia, similar

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to the method used by Buinauskas and associates(1). The tumor used was the Walker 256 carcinosarcoma, injected subcutaneously.

The plan of the experiment was to perform a celiotomy on groups of 15 to 30 rats. These celiotomies were performed from 1 to 30 days before injection of tumor cells was made. In this manner a uniform stress was induced at variable intervals before tumor injection. Also in this manner we were able to use the same tumor suspension for each experimental group of animals. Because of the very large number of animals involved it was necessary to repeat the experiment 4 times.

Celiotomy was performed on 15 to 30 rats daily for 8 days, then on alternate days, through a period of 30 days. Each rat was anesthetized with 30 mg of Nembutal per kilo of body weight injected intraperitoneally. The abdomen was then shaved and the skin prepared for aseptic technique. Under sterile technique a midline incision 3 to 4 cm in length was made. The wound was left open for 30 minutes; the intestines were kept moist with physiologic saline at intervals of about 5 minutes. After this 30-minute period the viscera were replaced and the abdominal wall was closed in 2 layers with 4-0 white cotton. The rats were then replaced in their cages to await the appropriate interval for injection of tumor cells.

Thirty days after the first group of animals were operated on, all groups, including a non-operated control group, were inoculated with tumor cells. Accordingly, the last group of animals operated on received tumor cells on the same day as the operative stress; whereas the group operated on first, received tumor cells 30 days after the operative stress.

The suspension of Walker tumor cells was made according to the method previously used in this laboratory. After excision of the tumor under sterile conditions, the tumor was minced with scissors and passed through a steel wire mesh screen (80 mesh per inch) to obtain a finely divided tumor suspension. The tumor suspension was then allowed to stand at room temperature under sterile conditions for 8 hours, according to the method of Chan and associates(3). This aging is done to reduce the number of takes in the control groups to 50% or thereabout. After the aging process the cell suspension is counted in a WBC counting chamber; proper dilution with sterile isotonic saline was made to obtain a viable cell count of 20,000 cells per cc. All handling of the tumor suspension was done under sterile conditions. The tumor inoculum was injected subcutaneously in the abdomen of each animal in a volume of one cc. The rats were inoculated successively, one at a time from the various groups including a control group so that the average time representing age of the cell suspension would be the same for all groups including the control group.

This experiment was repeated 4 times, but included more animals in the groups from 6 days to 22 days following celiotomy because this was obviously the most important period. In this period the smallest number of animals in any one group was 48 and the largest 64.

After injection of the tumor cells the groups were observed for takes twice a week the first 4 weeks, and once a week after this for a total of 3 months.

Results. The tumors began to appear 10 to 14 days after inoculation and appeared in all groups at approximately the same time. Our main interest was in the resistance of the animals to the injected cells. Thus, we were particularly concerned with the percentage take in the experimental groups as opposed to that in the control group.

The takes reported herein are those noted 6 weeks after tumor injection. By this time the tumor take had stabilized, and the count remained constant in the ensuing weeks. As would be expected following previous experiments in this laboratory the highest incidence of tumor take occurred in those animals having the shortest interval between celiotomy and tumor injection. Thus the highest incidence (90%) of tumor take is in those rats which had the celiotomy on the same day as the injection of cancer cells. The incidence of tumor take decreased to the control level at day 10, but continued to decrease until the 18th day (Fig. 1). The difference in takes at days 16 and 18 as observed in the

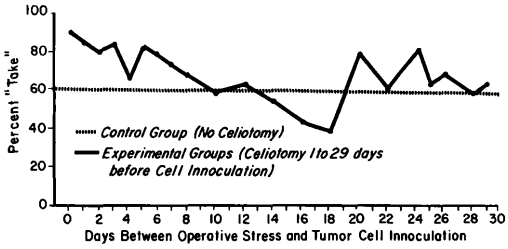

FIG. 1. Relationship of take to time interval between stress (celiotomy) and tumor cell inoculations. Note that when cells were inoculated immediately after stress the takes were 90%, indicating a very low resistance. However, the resistance increased gradually (with a lowering of % takes) for 6 or 7 days; but the resistance continued to increase gradually beyond that of the controls until the 18th day when the takes were only 41% compared to a control level of 60%. Between the 18th and 20th days the resistance decreased, but returned to normal a few days later (by the 28th day).

experimental groups (44 and 41% respectively), as compared with the control group (60%) is statistically significant ($p = .02$). The percent take then rose to a level of 79% in the animals inoculated 20 days after celiotomy. Following this the percent take decreased gradually, returning to normal in the group inoculated with cells 28 days after celiotomy.

Discussion. We have no explanation for this increase in resistance to cancer cells observed 16 to 18 days after celiotomy, other than the supposition that it is related to the celiotomy. Moreover there is no definite explanation of the reduced resistance to cancer cells found so consistently for 2 to 5 days after celiotomy. At first it was thought that it was related to the adrenal response observed in the Selye stress phenomenon. However, the work of Slawikowski(9) suggests strongly that neither the adrenal nor hypothesis exerts a significant influence on this reaction; at least their ablation did not prevent the production of decreased resistance to cancer cells after celiotomy. It is very possible that the liver is a major factor in the phenomenon.

Summary. Approximately one thousand Sprague-Dawley white rats were subjected to the stress of a celiotomy at various intervals (0 to 30 days) before inoculation with Walker 256 tumor cells. The low resistance noted when cells were injected at the time of stress (as measured by a take of 90%) increased gradually from day to day reaching the control value (60%) by the eighth day, but continued to show an increased resistance for several more days, with a take of only 41% on the 18th day. The resistance decreased rather sharply to a take of 79% on the 20th day, coming back to the control level of 60% within the next few days.

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Respiratory Catabolism in Man of the Degradative Intermediates of L-Ascorbic-1-C^{14} Acid. (28371)

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Earlier studies have shown that there is little or no oxidation of the carboxyl carbon of L-ascorbic acid to CO_2 in man(1,2). Recent experiments showed that rapid decomposition of L-ascorbic-1-C^{14} acid occurred when a dilute solution of it remained at room temperature in the dark for 24 to 72 hours. When this material was given to either ani-