ever, transfused cells in 3 rats had reached low levels by 15th day. Immunization against C erythrocytes was not indicated since a secondary response was not elicited by a second or third transfusion of C type cells. The CD strain of rats also segregate for independently inherited red cell antigens (E and F) both of which are isoantigenic (R. D. Owen, personal communication). It is possible that shortened survival in the 3 rats of the first experiment resulted from production of antibodies against these or other unidentified red cell antigens. Failure to observe "immune clearance" in the second and third transfusion may then reflect the absence of corresponding isoantigens in donors.

Our data suggest that the Cr^{51} method gives a reliable estimate of life span of rat erythrocytes when the extinction point is used, although the method gives a falsely shortened 50% survival time because of chromium elution. The life span we obtained compares well with that reported by Berlin *et al.*(13) and Owen (personal communication) but is considerably longer than that reported by Hall *et al.*(1). Differences in technic could partly account for the variance since these workers obtained the first blood sample 1 hr after transfusion, whereas we used a 24-hr period. The relative merits of each method are discussed by Eadie and Brown(11).

Summary. Survival of rat erythrocytes has been studied by simultaneous use of 2 methods, Cr^{51}-labeling and differential agglutination. Results suggest that random destruction of rat erythrocytes did not occur and that the extinction of radioactivity of Cr^{51}-labeled cells is a valid criterion for determination of erythrocyte life span. Both methods suggested a life span of about 65 days.

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Immunologic Treatment of Tumors.*† (24513)

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We have prepared anticancer antibodies by injection of tumor cells into animals of a different species, and have injected the antibody solution into host bearing a tumor. Antibodies prepared in rabbits against Walker 256 rat carcinosarcoma were injected into rats at the same time they were inoculated with 10,000 Walker 256 cells. The recent work of Hiramoto and Nungester(1), Korngold and van Leenwen(2) among others showed the feasi-

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bility of producing antibodies against certain tumors; Korngold and associates showed the reactive sites *in vivo* by means of electrophoretic and isotopic studies. It was believed that isolation of large amounts of concentrated gamma and alpha-2-globulins prepared specifically against tumor substances by immunologic techniques might prove of value in treatment of cancer.

Technic. Anticancer cell antibodies were prepared against Walker 256 rat tumor by inoculating several million Walker cells into rabbits every 4 days for 3 or 4 injections, and subsequently fractionating gamma and alpha-2-globulins from their serum. Cancer cell antibody titer was then determined and if adequate, the animals were exsanguinated. As antibodies were also formed against the small amount of normal tissue not removed, these were absorbed out by modification of technic of Edwards and Ewing(3). Serum containing the antibody was kept overnight at cold temperature on an electro-magnetic stirrer, and enough of a single cell suspension of normal tissue was added to allow complete absorption of "normal" tissue antibody. The serum was then centrifuged and tested for specificity. Gamma and alpha-2-globulins were isolated by modification of Cohn's(4) "5-point variable" alcohol precipitin technic as adapted by Nitschmann *et al.*(5), and further modified to pH to correspond to animal's blood. Thirty rats were injected subcutaneously with 10,000 Walker 256 cells. Twenty were injected with antibody serum prepared as described above and 10 were controls.

Results. Immunologic studies using the agglutination technic showed that the protein fraction containing gamma and alpha-2-globulins had more than 100 times the amount of antibodies found in antiserum before it was fractionated. It was found that adsorption of normal tissue antibodies did not remove cancer cell antibodies and that adsorption technic used was effective in removing the antibodies formed against normal tissue.

In all 10 control rats, "takes" were palpable on eighth day. In 20 animals injected with antibody serum, "takes" were prevented in 12; in the remaining 8, "takes" were not demonstrable until 22nd to 25th day. The animals were observed for additional 90 days but no more "takes" were noted. It was also found that this protein fraction did not give rise to anaphylactic or toxic reactions. When the protein fraction was injected systemically into rats with previously established tumors the growths decreased in size by about one quarter but commenced to grow again after about 48 hours. The tumors in treated animals remained smaller than in controls. Direct injection of the anticancer fraction into the tumor did not appear to be more effective than its administration systemically. Injection of normal rabbit gamma and alpha-2-globulins had no effect on the tumors.

Two patients suffering from advanced cancer of the breast with supraclavicular node metastases have been treated with gamma and alpha-2-globulins isolated from sheep, which had received 6 injections of 15 million cancer cells obtained from the primary breast tumor of the patient. Three animals were used for each patient. In one patient the supraclavicular metastatic node measured 4×3 cm before injection of the antibody serum, and 24 hours after injection of the serum it measured 3×2 cm. In the other patient the supraclavicular node measured 7×4.75 cm before injection, and 24 hours after injection measured 5.6×3.75 cm. In neither case was any diminution in size noted during succeeding days. Immunologic studies showed that gamma and alpha-2-globulins had a high titer against cancer cells. In one patient a piece of cancer tissue removed after treatment did not react with the protein fraction whereas it did react prior to treatment, signifying that antibody was "coating" the cancer tissue. Following injection, one patient developed a chill, and the other, pain in lumbar region after injection, but in neither case could these reactions be considered of major significance.

Summary. We produced antibodies by injecting cancer cells obtained from Walker 256 tumor into rabbits; after several injections of the cell antigen, the rabbits were exsanguinated, and the serum fractionated. Antibodies to normal tissue were adsorbed out by expo-

sure to normal tissue. Injection of the globulin fraction prevented "takes" of Walker 256 cells in slightly over half of rats inoculated with 10,000 cells. The globulin antibody solution produced a decrease in size by about one quarter when given to rats with tumors 2 to 3 cm in diameter, but the tumor masses did not disappear. The method was tried in 2 patients with decrease in size of the tumor but not disappearance.

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Effect of Thyroidectomy on Pancreatic Amylase.* (24514)

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Hypophysectomy of the rat induced a striking reduction in weight of the pancreas (1,2) and in size and granulation of acinar cells(3). Also, amylolytic(1,4), proteolytic (5) and lipolytic (unpublished) activities were depressed significantly. That some of these changes may have resulted from deficient thyroid secretion was indicated by the occurrence after thyroidectomy of partial pancreatic atrophy(6) and reduced total lipolytic activity. Furthermore, morphological evidence of stimulation appeared following administration of thyroid substance to nonoperated rats(7-11). The purpose of our experiments was to observe the effect of thyroidectomy on pancreatic amylase. If the direction and magnitude of change were similar after both hypophysectomy and thyroidectomy, additional evidence would be provided to support the hypothesis that a significant portion of hypophyseal regulation over enzyme production by the pancreas is mediated by the thyroid gland.

Methods. Young adult female rats of Sprague-Dawley strain were used, with the following treatments given to one-half of the rats in each experiment: Exp. 1, surgical thyroparathyroidectomy; Exp. 2, parathyroidectomy by electrocautery; and Exp. 3, surgical thyroparathyroidectomy in addition to 100 μc of I^{131} subcutaneously 13 days later. Radioactive iodine was given to the third group to insure destruction of thyroid fragments which are often left after surgical thyroidectomy. Completeness of gland removal was verified by microscopic examination of the cervical region in all rats of Exp. 1 and in some of Exp. 3. Those which retained fragments of significant size were eliminated. Exp. 2 acted as a control for Exp. 1 in that an opportunity was provided to observe the effect of parathyroidectomy alone. After operation the controls were pair-fed against the operated rats. The animals in Exp. 1 and 3 were fed "Low Iodine" Test Diet (Nutritional Biochemicals Corp.). To this was added 1 g glucose/100 g of test diet for operated animals, and the same amount of glucose in addition to 3.27 mg of KI for control animals. Animals in Exp. 2 were fed powdered Purina Laboratory Chow. All rats were given water *ad lib.* but no food for 24 hours prior to termination of experiment. After killing the rat by blow on head, the pancreas was excised,

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