uptake showed that absorption, diffusion and utilization of the combined fat emulsion were possible and effective through the subcutaneous pathway. The comparative studies indicated a parallelism with that obtained by the intravenous route.

The cause of temperature elevation and change in oxygen uptake in conjunction with clyses of the emulsion cannot be explained fully. The latter may be accounted for on the basis of a thermogenic response due to increased heat production associated with the combustion of fat. These changes may also be considered to be due to the presence of pyrogens in the emulsion. Should this be the case then the latter must be present in the coconut oil since the other ingredients have been proved to be pyrogen free prior to emulsification. Nevertheless the present combined fat emulsion is still being used clinically while these aspects of the problem undergo further investigation.

Summary. (1) A liter of a 10% combined fat emulsion was injected subcutaneously into hyaluronidase treated areas in each of 22 patients. (2) Each of 8 of the latter received the combined fat emulsion both by the intravenous and the subcutaneous routes. (3) Six patients in this series received 3 consecutive hypodermoclyses of the emulsion. (4) The general clinical reactions and the changes in blood lipids, urinary lipids and oxygen consumption were studied. (5) The clinical effectiveness of this type of administration for parenteral fat was established.

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Effects of Enzyme Inhibitors on *in vitro* Dehydrogenase Activity of Cancer Tissue Slices.* (18864)

MAURICE M. BLACK, ISRAEL S. KLEINER, AND FRANCIS D. SPEER.

From Departments of Pathology and Biochemistry, New York Medical College, Flower and Fifth Avenue Hospitals, New York.

During an investigation of the histochemical appearance of mouse tumor and control tissues incubated with TTC in the presence and absence of certain enzyme inhibitors it was found that these agents did not inhibit the reduction of 2,3,5-triphenyl tetrazolium chloride (*TTC*) by the tumor slices while inhibition of the control tissues was observed (1). It, therefore, appeared desirable to evaluate quantitatively the effects of several enzyme inhibitors on reduction of TTC by diverse human control and cancer tissues. Our findings indicate that under the experimental conditions employed fluoride causes less inhibition of the dehydrogenase activity of cancer tissue slices than it does in the homologous control material. Cancer tissue also shows minimal inhibition by malonate, but such findings may also occur in some normal tissues.

Materials and methods. Samples of human control and cancer tissues were obtained from surgical specimens within a half hour of the time of operation. The mouse tumors studied included spontaneous breast carcinomas arising in CFW and C3H strain mice as well as a transplantable mammary carcinoma grown in C57 black mice. Tissue slices, approximately 1 mm in thickness were prepared and placed in individual wide mouthed tubes containing the appropriate incubation mixtures, each of which contained 3 ml of a 1% aqueous solution of TTC buffered with phosphate buffers to pH 7.2. The different mixtures had in addition one ml of (a) 0.9% sodium chloride

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TABLE I. Reduction of TTC by Normal and Tumor Tissue With and Without Added Enzyme Inhibitors.

Tissue	Diagnosis	No. Cases	μg R_O	TTC R_F	Reduced/mg R_M	Reduced/mg R_{N_3}
Breast, human	Control	8	.8	.6	1.2	.4
" "	Carcinoma	28	.6	.6	.8	.5
" mouse	" *	19	1.3	1.3	1.5	1.4
Stomach	Mucosa	11	1.2	.7	1.1	1
"	Carcinoma	5	.1	.1	.2	.2
Colon	Mucosa	11	1	.5	.9	.6
"	Carcinoma	11	.7	.5	.7	.4
Kidney	Cortex	6	2.8	1.4	1.8	—
	Carcinoma	1	2.3	5.1	2.7	1.5
Lymph node	Control	1	.3	0	.7	—
	Acute leukemia	1	1.2	1.9	1.9	1.6
Thyroid	Control	15	.4	.3	.7	.3
	Carcinoma	2	.7	1.1	2.2	.9
Testicle	Control	1	4.5	4	4.5	4.5
	Embryonal carcinoma	1	1.1	1	1.2	.9
Metastatic ca.	Primary site unknown	3	1.1	1.3	1.3	1
Bone	Ewing's tumor	1	.7	1.1	.7	.6
Skin	Melanoma	1	.5	.8	1	.3
Retroperitoneal granuloma	Non-specific	1	.6	0	0	—
Sacrum	Teratoma	1	1.1	1.1	2.4	1

* Data includes spontaneous breast cancer in C3H and CFW mice as well as a transplantable breast carcinoma.

(Ro), (b) 10^{-3} M sodium chloride (R_F), (c) 10^{-3} M neutralized malonic acid (R_M), and (d) 10^{-3} M sodium azide (R_{N_3}). The tubes were placed in a rack in an incubator, maintained at 37°C and subjected to continuous gentle agitation for a period of one hour. At the end of this time they were removed, the solutions decanted and subjected to acetone extraction of the reduced TTC. The concentration of reduced TTC in terms of μg per mg of acetone dried tissue was determined according to the technic previously described(2,3).

Results. In Table I we have indicated the extent of the TTC reduction by the various tissue slices incubated in the different experimental media. Where they were available we listed the homologous control and cancer tissue together. These data show that fluoride, in the concentrations employed, caused decidedly less inhibition of the cancer tissue than of the homologous controls (Fig. 1). In many instances actual increases in TTC reduction by cancer tissue slices were observed, particularly in the kidney carcinoma. Whereas the TTC reduction by inflammatory granulomas is markedly depressed or abolished, this is not true of fibrosarcoma tissue slices. This latter statement is based on histochemical observations of fibrosarcomas. Moreover, while carcinomatous tissue tends to show a slightly reduced endogenous dehydrogenase activity as compared to the control material, the reverse is true in sarcomas. Thus, normal lymph nodes and stromal tissue show only minimal reduction of TTC while lymphosarcoma and fibrosarcoma tissue are intensely stained. In common with carcinoma, such staining is not inhibited by fluoride under the experimental conditions employed.

There appears to be a linear relationship between the endogenous value and the amount of TTC reduced in the presence of fluoride (Fig. 2). While the relationship holds in the control and in the tumor tissues, the curves are separate and show increasing divergence with increase in the endogenous dehydrogenase activity. Although the individual values for the endogenous dehydrogenase activity within a particular tumor type may vary, the relationship to fluoride tends to remain constant. In Fig. 3 we have plotted

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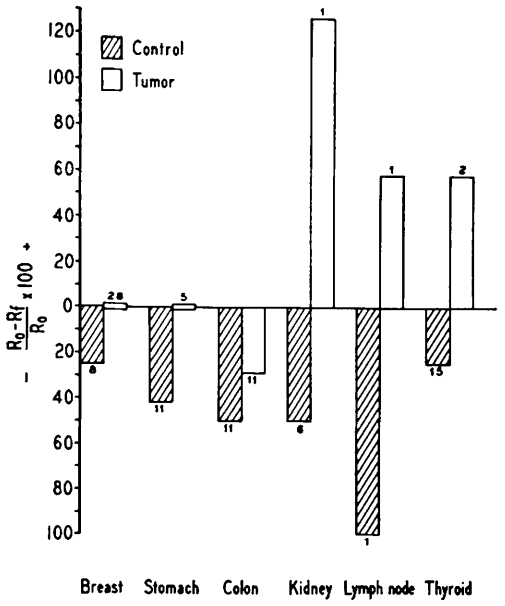

FIG. 1. % change in TTC reduction produced by NaF in homologous cancer and control tissue slices. Values in bars indicate No. of cases in each group.

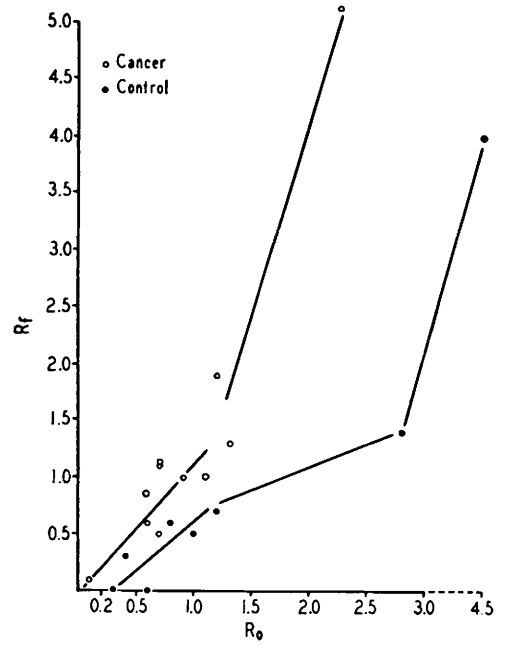

FIG. 2. Relationship between mean values for endogenous R_o and R_F reduction in control and cancer tissues.

such individual values for human and mouse mammary carcinoma; the same straight line relationship is obtained both for the human and mouse tissues.

Under the conditions employed, malonate failed to inhibit the various tumors and in many cases was associated with increased TTC reduction. However, some of the control tissues showed similar findings. In the experiments with sodium azide, the control and tumor tissues tested showed inhibition of varying degrees.

Special note should be taken of the findings in the mammary carcinoma of mice since these tumors tend to present a more uniform tumor cell population than obtains in human cancers. The absence of inhibition by fluoride, malonate or azide confirms our previous report based on frozen section examinations of the tissue slices(2). These findings were the same in spontaneous breast tumors arising in CFW and C3H mice as well as in a transplantable breast carcinoma grown in C57 black mice. This suggests that the data under discussion is not appreciably influenced by the host but reflects activity indigenous to tumor metabolism.

Discussion. The reduction of TTC by tissue slices provides a measurement of the dehydrogenase activity of viable tissues. While the particular substrates being metabolized during endogenous tissue respiration are not known, the effects of selected inhibitors of

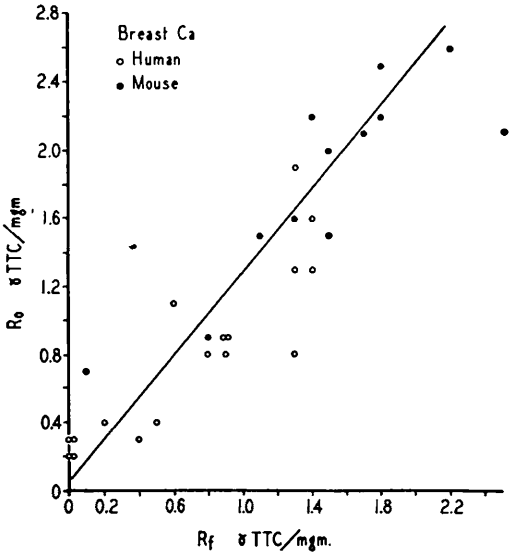

FIG. 3. Relationship between R_o and R_F values in individual cases of human and mouse mammary carcinoma.

enzyme activity on the TTC reduction may serve to characterize the type of metabolism in so far as such effects are reflected in oxidation-reduction reactions. Thus the experimental data presented may be considered in terms of pertinent information in the literature on biochemical features of cancer tissues. Among these are (a) tumors possess an increased aerobic glycolysis as compared to most normal tissues(4); (b) glycolysis in tumors appears to follow the Embden-Meyerhof schema(5-7); (c) tumors show minimal activity in regard to the citric acid cycle of reactions(8,9).

It has been shown that sodium fluoride may affect glycolytic reactions in two ways: (1) by inhibiting enolase and thus preventing the transformation of phosphoglyceric acid to pyruvic acid and (2) by inhibition of phosphatase activity. Since phosphatases may remove the phosphate from hexose diphosphate and other phosphorylated hexoses, they may limit the metabolic degradation of such compounds.

The enolase inhibition by fluoride would result in a decreased pyruvate formation. In so far as pyruvate is required to function as a H acceptor in the regeneration of oxidized coenzyme, previously reduced by the oxidation of phosphoglyceraldehyde to diphosphoglyceric acid, decreased available pyruvate would limit subsequent oxidation. Since it is mediated through a dehydrogenase such an effect should result in decreased TTC reduction. However, while most normal tissues remove pyruvate for participation in the reactions of the Krebs cycle, this occurs to a lesser degree in cancer tissue, thus minimizing the limited effect of enolase inhibition by fluoride. Moreover, the inhibiting effect of fluoride on dephosphorylations by phosphatases would provide more available substrate for triose formation and oxidation. From these considerations fluoride should result in an increased phosphoglyceric acid formation, and TTC reduction in cancer tissues. Novikoff *et al.* show in their Fig. 7 and 8 that fluoride produces such a change in homogenates of tumor tissue(5). Thus the increased TTC reduction found by us to occur in tumor tissue in the presence of fluoride is consistent with the Embden-Meyerhof schema of glycolysis and the known effects of fluoride on this mechanism. As judged by the general similarity of the curves shown in Fig. 2 a parallel relationship obtains in control and tumor tissues, suggesting that cancer tissue does not possess a different type of metabolism than is operable in most normal tissues.

It should be emphasized that we do not infer that the biological phenomenon of malignant neoplasia has been defined by quantitative determinations of the type reported or referred to here. The rapidly proliferating tissues of the normal testis and benign teratomatous proliferations may also show minimal fluoride inhibition under the conditions employed (Table I). While the data presented in the control and homologous tumor tissues appear to be related to the property of continued growth in the tumors, it cannot be said to reflect another fundamental property of cancer tissue, namely, metastatic potentialities. Biochemical representation of this phenomenon is still lacking and very much needed. This accords with our previous report on the features of morphology and metabolism in malignant neoplasia(10).

Summary. 1. Tissue slices from various human cancer and control tissues and mouse breast cancer were incubated in TTC solution with and without the enzyme inhibitors: fluoride, malonate and azide and the μg of TTC reduced/mg of acetone dried tissues determined. 2. Both control and tumor tissues exhibited a linear relationship between the endogenous and fluoride inhibited TTC reduction. The tumors were characterized by

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a decreased fluoride inhibition as compared to homologous controls. Malonate inhibition of tumor tissue also tended to be minimal. The findings were discussed in terms of the metabolism of tumor tissue.

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Adrenal Cortex and Peptidase Activity of Rat Tissue.* (18865)

FRANK L. ENGEL AND THEODORE B. SCHWARTZ.†

From the Department of Medicine, Duke University School of Medicine, Durham, N. C.

There is general agreement that overactivity of the adrenal cortex results in a net increase in protein catabolism. This may result from a decreased rate of protein anabolism, an increased rate of protein catabolism, or both mechanisms may be operative(1). If the rate of protein breakdown were appreciably increased by adrenal cortical stimulation, a concomitant enhancement of proteolytic enzyme activity might not be unexpected. Therefore, an investigation of the effect of ACTH administration on the activity of several peptidases of various rat tissue homogenates and extracts was undertaken.

Materials and methods. Tissue extracts were prepared essentially as described by Smith(2). After a 48-hour fast, adult male rats of the Vanderbilt strain were anesthetized with nembutal, exsanguinated through the aorta, and one or more of various tissues were removed, rapidly weighed and transferred to a Waring blendor containing crushed ice and water. After mincing for 2 minutes, an aliquot of the homogenate was diluted with an equal amount of 0.02 M barbital buffer at pH 7.8 and refrigerated. The remaining homogenate was spun at 12,000 RPM for 30 minutes in a Servall centrifuge in a cold room at 4°C. An aliquot of the clear supernatant solution was similarly buffered at pH 7.8. Peptidase activity of these buffered mixtures was determined within a few hours after they were prepared. A modification of the procedures of Beloff and Peters(3) and Fruton(4) was used in preparing crude extracts of skin. About 5 g of skin was removed from the abdominal wall of an anesthetized rat. After subcutaneous tissue was scraped off, the skin was cut into strips about 2 mm wide. A coiled strip of skin was placed on a freezing microtome and the 50 μ sections taken were placed in 10 ml of 2% NaCl. The suspended sections were stirred for 3 hours at room temperature and then centrifuged at moderate speed for 30 minutes. Peptidase activity was determined on an aliquot of the clear supernatant solution. This entire procedure was carried out on skin obtained from 2 separate sites from each animal. The nitrogen content of unbuffered homogenate, supernate, and weighed portions of oven-dried whole tissue were determined by a semi-micro-Kjeldahl technic. Peptide hydrolysis proceeded in 10 ml Erlenmeyer flasks continuously shaken in a water bath maintained at 38°C. The hydrolysis mixture consisted of 0.5 ml of tissue preparation, 0.5 ml of 0.1 M substrate, 0.5 ml of 0.02 M barbital buffer (pH 7.8), and 0.5 ml of 0.85% N Cl. Hydrolysis mixtures containing glycyl-glycine as substrate were made 0.001 M with respect to cobalt. At 1, 2, and 18 hours, 0.2 ml samples were taken from the

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† Public Health Service Fellow of the National Institutes of Health, Public Health Service (1950) and Damon Runyon Senior Clinical Research Fellow of American Cancer Society (1951).

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