the pituitary or only transmits tracts from other centers is also not clear.

The results of certain investigators with pituitary stalk-sectioned rats is compatible with the data obtained in this investigation. Brolin(9) found that the usual marked hypertrophy and hyperplasia of pituitary basophiles following thyroidectomy did not occur in stalk-sectioned rats and Greep and Barrnett observed a markedly decreased goitrogenic response following 2-3 weeks of thiouracil feeding in intact, stalk-sectioned rats(10). The latter authors, however, found that a marked infarction of the pituitary, similar to that seen in rat CR-1 of the present investigation, was produced by their technic of sectioning. Brolin did not state whether or not pituitary damage had been produced.

9. Brolin, S. E., *Acta Physiol. Scand.*, 1947, v14, 233.

10. Greep, R. O., and Barrnett, R. J.; Personal communication.

Summary. Bilateral, symmetrical, electrolytic lesions in the hypothalamus between the suprachiasmatic and caudal ventromedian nuclei prevented the goitrogenic response of the rat to thiouracil feeding. The iodide-concentrating capacity of the thyroid, however, increased to approximately the same extent as that of intact rats similarly treated. It appears that hypothalamic lesions of this type impair the secretion of thyrotrophin by the pituitary. It is not known whether this interference with thyrotrophin secretion is qualitative or quantitative, nor whether hypothalamic control is exerted by neural or hormonal mechanisms. The available evidence points to the secretion of a hypothalamic hormone, however.

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Subcutaneous Administration of Combined Fat Emulsion with Hyaluronidase.* (18863)

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Hyaluronidase has been proved to be an important adjuvant for the rapid absorption of a wide variety of fluids injected subcutaneously. The principles and the technics for the introduction of fluids into the body by means of hyaluronidase have been applied in the present study to the administration of a combined fat emulsion developed in our laboratory. This emulsion has been previously reported upon with regard to its clinical effects when infused intravenously into human subjects(1,2).

* Aided by a grant from the Abbott Research Laboratory.

1. Shafiroff, B. G. P., Mulholland, J. H., Roth, E., and Baron, H. C., PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 343.

2. Shafiroff, B. G. P., and Mulholland, J. H., *Ann. Surg.*, 1951, v133, 145.

Method of study. Hyaluronidase and the combined fat emulsion were infused subcutaneously into 22 patients. Each subject received one liter of the 10% combined fat emulsion. The latter contained 100 g of purified coconut oil, 50 g of the amino acids contained in commercial intravenous protein hydrolysate solution, 50 g of dextrose, and 30 g of gelatin as the stabilizing agent. It provided approximately 1200 calories per liter and the particle size of the emulsion averaged 1 μ. Hyaluronidase was injected immediately prior to the infusion in doses of 500 units into the anterior aspect of each thigh about 6 inches above the knee. The emulsion was infused into the subdermal tissue at rates varying from 4 ml to 7 ml per minute. All studies were begun with each patient in the basal or postabsorptive state and continued after the

TABLE I. Summary of Data of Patient J.H. Who Received One Liter of the 10% Combined Fat Emulsion as a Clysis.

	Time in hr	Temperature, degrees F	Total lipid in blood serum, mg/%	Urine volume, ml	Total lipid in urine sample, mg
	−2	98.2	542	150	.6
	−1	98.8	475	—	—
Infusion period	0	99	350	72	7.7
	1	99	507	51	5.6
	2	99.2	623	22	2.6
	3	98.8	628	18	16
	4	98.6	725	16	3.2
	5	99	698	22	7.3
	6	98	634	26	.4
	7	100	—	31	1.6
	8	100	670	—	—
	9	100	—	—	—
	10	99.4	498	—	—
	11	100	—	94	.4
	12	100	530	—	—
	13	98.4	—	—	—
	14	99	460	103	6.4
	15	99	—	—	—
	16	99	—	—	—
	17	99	—	89	13.4
	18	98	464	—	—
	19	98.8	—	—	—
	20	98.8	—	90	3.5
	21	98.6	428	—	—
	22	98.6	442	—	—
	23	98.8	—	102	.7
	24	98.8	442	—	—

infusion through a period of 24 hours. Clinical records of temperature, pulse, blood pressure and respirations were taken at hourly intervals. Blood samples were taken on a regular schedule for the quantitative determination of total lipids(3). Urine samples were collected in fractional portions throughout the day and analyzed for their fat content(3). Quantitative estimations of oxygen uptake were made serially. Biopsy sections were taken through the infusion site for histo-chemical examination. For comparative purposes 8 of the patients in this series were also infused intravenously with the same type emulsion and identical studies as those above were made.

Results. The subcutaneous infusion of one liter of the 10% combined fat emulsion was well tolerated by all of the patients in this series. In 86% of the subjects there was a temperature elevation of one to 2 degrees above the initial pre-infusion temperature. In 6% the rise exceeded 2 degrees while in the other 8% the elevation was less than one degree. In all the subjects the temperature returned to its usual pattern at the end of the study period. There were no significant changes in blood pressure. Total blood lipids reached a peak level 30 minutes to 4 hours after the beginning of the clysis, varied in each case but usually attained the highest concentration early in the post-infusion phase of the study. The peak blood lipid value generally averaged twice that of the fasting level. At the end of the study period blood lipid values returned approximately to the initial levels in all cases. The rate of decline of total blood fat from the peak level varied in each case. The total amount of lipid recovered from the urine averaged 10 to 15 mg. One patient, a paraplegic, with severe kidney disease excreted a maximum of 69 mg of urinary lipids after the infusion of the emulsion. Oxygen consumption rose slowly during the course of the emulsion clysis, reached a peak level in the post-injection period and then tended to return to the initial level. The average maximum increase in oxygen uptake was 20%.

3. Bloor, W. R., *J. Biol. Chem.*, 1928, v77, 53.

TABLE II. Data from Same Patient J.H. Who Received Also One Liter of the 10% Combined Fat Emulsion Intravenously.

	Time in hr	Temperature, degrees F	Total lipid in blood serum, mg/%	Urine volume, ml	Total lipid in urine sample, mg
	−2	99	490	—	—
	−1	98.4	500	90	5
Infusion period	0	99	500	—	—
	1	100	570	—	—
	2	100	580	75	4.3
	3	100	678	50	6.6
	4	100	600	45	15.7
	5	100	—	—	—
	6	98.6	510	—	—
	7	100	515	60	5.1
	8	99.8	450	—	—
	9	99.2	408	170	3.5
	10	98.2	410	—	—
	11	98.8	—	200	2.4
	12	98.6	—	—	—
	13	98.4	420	—	—
	14	98.4	—	180	2.5
	15	98.8	—	—	—
	16	98.8	410	—	—
	17	98	440	75	.9
	18	98	520	—	—
	19	98.2	—	—	—
	20	98.2	500	98	.6
	21	98.4	—	—	—
	22	98.6	—	—	—
	23	98.6	—	—	—
	24	98.6	500	20	.8

No evidence of a toxic systemic reaction was noted nor was there any pathology at the site of injection that could be attributed to any of the constituents of the emulsion. The biopsy sections which were taken on the third post-infusion day were negative for tissue necrosis, granulomata or residua of fat. The latter observations were made from sections specially stained with Sudan III and oil-red-O. Included among the latter were biopsy sections from subjects who had received three consecutive clyses of the emulsion. Subjectively, the majority of the patients reported a sensation of warmth in the injected extremities and complained of tenderness to pressure over the infusion sites. Both of these symptoms subsided spontaneously within 2 or 3 days.

In the comparative tests with infusion by the intravenous route, temporary elevations of temperature developed similar to that previously described. Total blood lipids attained a higher peak level than that observed after hypodermoclysis and the curve of the rate of disappearance of fat from the blood seemed to follow a more constant pattern. Excretion of urinary lipids usually averaged less than 10 mg for the study period except for the paraplegic previously mentioned who again showed a greater loss of fat in the urine than the others. A progressive rise in oxygen uptake was found to occur more rapidly and the increments of increase were generally higher than those obtained after hypodermoclysis of the emulsion.

Discussion. This study has shown that the combined fat emulsion together with hyaluronidase can be successfully administered for nutritive purposes via the subcutaneous route. The facility for absorption of the combined fat emulsion can be accounted for by the action of hyaluronidase and its enzymatic hydrolysis of hyaluronic acid. Without hyaluronidase subcutaneous deposits of the emulsion were poorly tolerated in large amounts and were absorbed very slowly. Increase of total lipids in the blood, excretion of lipids in the urine and changes in oxygen

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)

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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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