mination have been used to characterize the partially purified acid mucopolysaccharides obtained from urine of normal persons, from patients with gargoylism and from their relatives. The results, expressed as a ratio of carbazole to naphthoresorcinol hexuronic acid, demonstrated a similar abnormal qualitative excretion of chondroitin sulfate B by patients with gargoylism, by one or both parents, and by some of their other relatives. Occurrence of an abnormal qualitative excretion of chondroitin sulfate B in the clinically normal relatives of patients with gargoylism probably indicates the heterozygous carrier state of the disease.

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Rate of Fat Uptake by Intestinal Lymphatics.* (26914)

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The time course of fat absorption has been studied most frequently by following the changes in blood lipid concentration at intervals after fat meals using either the chylomicrograph(1,2) or chemical methods(3) for analysis of plasma lipids. Disappearance of the excess lipid from the circulation after fat feeding has often been taken as an index of the completion of fat absorption, but this is based upon at least 2 important assumptions: 1) that the methods of measurement are sufficiently sensitive to detect extremely small amounts of absorbed lipid and 2) that the rate at which fat is added to the circulation during fat absorption always exceeds the rate at which it is removed. The time at which the excess lipid disappears following a fat meal is highly variable, ranging from 4 hours (1) to 10 hours(2) using either of the above methods. However, recent studies using the more sensitive I^{131}-labelled triolein technic have demonstrated the continued presence of I^{131}-labelled lipid in the circulation for periods extending beyond 24 hours after its oral administration(4) suggesting either a delayed removal rate or a prolonged absorption period. In the present study evidence will be presented to show that absorbed fat may continue to enter the circulation through the thoracic duct for 24 hours or more after a single oral fat load. Some of the reasons for this prolonged absorption period are examined and discussed in relation to the above mentioned studies involving the simple measurement of plasma lipid concentration during alimentary lipemia.

Methods. The study was carried out in dogs ranging in size from 10 to 18 kg, maintained on a nutritionally adequate "fat-free" diet for a variable period of time prior to experiment. This diet contained: purified casein, 24%; sucrose, 40%; corn starch, 16%; cellulose, 14%; salt mixture (U.S.P. IV), 4%; complete vitamin mixture, 2%;

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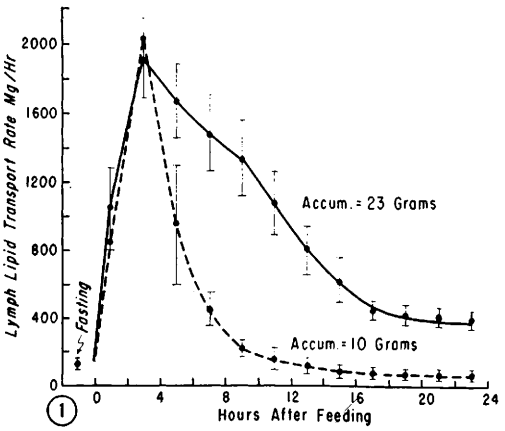
and was fed at the rate of 20 g/kg body weight/day. A fat-free diet was chosen because it was a later purpose to add known amounts of fat to this diet and to measure the rate at which the added fat was transported from the intestine through the lymphatic channels. The technic for lymphatic cannulation has been described(5). This operation was performed on the fasted animal under nembutal anesthesia, and the lymph was drained continuously into a flask which the animal carried. In 4 of the 15 dogs used, an indwelling lucite cannula was inserted into the duodenum to permit administration of the fat directly into the upper intestine. On the morning following the operation when the animals had fully recovered from the anesthetic, 25 g of olive oil were administered either by stomach or by duodenum. In the gastric feeding experiments, olive oil was either mixed and fed with the fat-free diet or administered alone by stomach tube. In the duodenal feeding experiments, olive oil was administered through the indwelling tube by syringe over a 5- to 10-minute period. In one case the animal was allowed to eat the fat-free diet immediately afterward. In all of the experiments lymph was collected at regular timed intervals (usually 2 hours) both prior to and following administration of the lipid. Post-feeding collections were continued for 24 hours. All lymph samples were measured for volume and aliquots were taken for total lipid measurement using the chloroform-methanol extraction procedure of Sperry and Brand(6).

In 2 separate experiments an effort was made to estimate the extent to which lipids absorbed through channels other than the thoracic duct might contribute to the lipids recovered from the lymph cannula. This was done because of the possible error inherent in the assumption that all of the excess lipid returned in the lymph after fat feeding is the result of a direct and unidirectional contribution from the intestine. Therefore, to test this assumption 2 thoracic duct fistula dogs were fed 50 g of olive oil and the lymph was collected until absorption was complete as evidenced by the visible clearing of the lymph on the following day. Three hundred to 400 ml of the milky citrated chyle obtained during the peak of the absorption period were then reinfused intravenously into the same dogs over a 2-hour period while continuing to drain the lymph from the thoracic duct. The extent to which the reinfused lipid escaped the vascular tree and appeared in the thoracic duct was estimated by measuring volume and total lipid in the thoracic duct lymph over 2-hour periods prior to, during and following infusion.

Results. The rate of fat transport in the thoracic duct is shown in Fig. 1. The mean fasting lymph lipid transport rate ± standard error is shown on the left for all dogs. This averaged 124 mg/hr after 24-48 hours of fasting and was independent of duration of the post-absorptive period. Attention is directed to the solid line representing the rate of lymph lipid transport in 11 dogs after receiving 25 g of olive oil by stomach. No apparent differences were noted between dogs receiving olive oil alone and those receiving olive oil mixed with the fat-free diet. Therefore, results from all stomach-fed dogs were pooled. Lymph lipid transport rate began to increase usually within the first hour after feeding and reached a peak whose average fell in the 2-4 hour interval. The time at which the peak occurred, however, was extremely variable, ranging from 3 to 9 hours. The maximum lipid transport rates achieved at the peaks were also highly variable averaging 1945 mg/hr but with a range of 1300 to 4800 mg/hr. Some of the animals exhibited sharp, highly elevated peaks early after feeding while others exhibited peaks which were less distinct, of lower magnitude and relatively delayed in time. This variability may perhaps reflect a variable stomach emptying rate among the different dogs. One other notable feature which the stomach-fed animals exhibited was an extremely prolonged absorption period, as evidenced by the fact that even at the end of 24 hours lipid transport rate was still significantly elevated above the average level found after 24-48 hours of fasting. In gross appearance the lymph still exhibited elevated turbidity 24 hours after a fat meal.

The dashed line of Fig. 1 shows mean rate



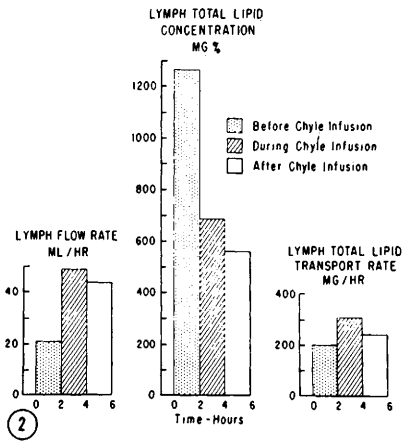


FIG. 1. Rate of fat transport by intestinal lymphatics. ●——● fed 25 g of olive oil by stomach at zero time. Mean of 11 dogs. ●– – –● fed 25 g of olive oil by duodenum. Mean of 4 dogs. Vertical bars represent stand. error. Accum. represents total 24 hr accumulated lipid transport.

FIG. 2. Flow rate and total lipid transport in thoracic duct lymph before, during and after intravenous infusion of 300-400 ml of homologous milky chyle. Mean of 2 dogs.

of lymph lipid transport in the dogs fed olive oil by duodenum. In this group the peak transport rate occurred in the 2-4-hour interval in all dogs. With respect to time at which the peak occurred and maximum rate of transport at the peak, the duodenum-fed dogs did not differ significantly from those fed by stomach. However, in the duodenum-fed group the transport rate had returned to the fasting level in approximately 13 hours. Statistical comparison of the stomach- and duodenum-fed groups from the time of the peaks to the end of the 24th hour revealed a significant difference at all intervals beyond the 7th hour ($P<0.01$). From these data it can be surmised that the prolonged absorption period in the orally fed dogs is in large measure the result of retention of fat in the stomach. The effectiveness of this retention factor in enhancing total absorption is revealed by the fact that the accumulated lymph fat output in the 24 hours was 23 g in the stomach-fed dogs and only 10 g in the duodenum-fed group.

That the recirculation of fat absorbed through other channels is not a significant factor in the delayed clearance is revealed by the experiments in which milky citrated chyle was reinfused intravenously into 2 thoracic duct fistula dogs in the post-absorptive state. In Fig. 2 it can be seen that the reinfusion process more than doubled the thoracic duct volume flow rate while almost halving total lymph lipid concentration. The total amount of fat transported increased slightly during the infusion period from 200 mg/hr to 309 mg/hr. Since the amount of lipid in the infused lymph averaged 19 g in the 2 dogs, this slight increase amounted to only 1.2% of the infused amount. This amount is probably negligible and serves to point out how effectively circulating lipids tend to be retained within the vascular tree or removed without any significant retransport in lymph.

Discussion. The results of the present study conflict with the widely held assumption that the process of fat absorption is completed within an interval of 10 hours or less following a single dose of fat by mouth. This assumption probably originated from experiments involving measurement of fat tolerance curves utilizing either chemical methods or the chylomicrograph to follow gross changes in blood total lipid concentration during absorption. It is unlikely, however, that these methods are sufficiently accurate to detect small changes in plasma lipids when the absorption rate is slow or when rate of removal of the lipid keeps pace with its rate of absorption. By measuring the lipid in the total volume of thoracic duct lymph prior to its entrance into the circulation one avoids the factor of utilization or removal and can conveniently and accurately measure both the time course of lipid

absorption and total amounts absorbed. It must be assumed, however, that the thoracic duct constitutes the major pathway for transport of absorbed lipids and that any lipid entering the circulation through other channels will not escape the vascular tree and recirculate through the lymph cannula. The first of these assumptions has been tested in a number of laboratories(7,8,9) and it is now generally held that at least 80% of long chain dietary fats are absorbed from the intestine through lymphatic channels and transported in the thoracic duct. The second assumption was tested in the present study, and it was shown that only 1.0% of the lipid in intravenously injected homologous milky chyle could be accounted for in the thoracic duct lymph during a 2-hour infusion period. This finding agrees with that of French and Morris(10).

In the present study more than 24 hours were required for the lymph to clear completely following a single oral dose of olive oil. In view of the above considerations it seems reasonable to conclude that this delayed clearance is the result of a prolonged absorption period. A large part of this prolonged absorption period would appear to be due to retention of fat in the stomach because when the olive oil was administered directly into the upper intestine the lymph cleared in approximately 13 hours.

The results in the stomach-fed dogs are at variance with those of Simmonds(11) in the rat. This investigator found that all of the measurable excess fat output in lymph occurred in the first 10 hours after administering olive oil through a gastrostomy tube. However, since only 0.5 ml of olive oil was fed, it seems doubtful that the increases in the lymph lipids would be high enough to be detected throughout the entire absorption period. In the human, Malm *et al.*(4) found 2-4% of a dose of I^{131}-labelled olive oil still present in the circulation 24 hours after feeding 5-10 ml of the labelled fat by mouth. The present study supports this finding and provides suggestive evidence that the continued presence of absorbed fat in the circulation after 24 hours is the result, at least in part, of a continuing absorption from the intestine. Part of this prolonged absorption period is probably the result of the slow delivery of fat by the stomach into the upper intestine, but this slow delivery perhaps serves in the best interest of the organism by permitting a larger percentage of dietary fats to be absorbed. Other evidence in support of the importance of the stomach for dietary lipid assimilation is provided by the common clinical finding that profound losses of dietary fat in the stool may occur following gastrectomy (12).

Summary. Measurements of the rate of fat uptake by the intestinal lymphatics showed that when dogs were fed 25 g of olive oil by mouth the total lipids in the thoracic duct lymph remained elevated above fasting levels for more than 24 hours. When the olive oil was introduced directly into the duodenum, the lymph was cleared of excess lipid in 13 hours. That the excess lipid in postcibal lymph probably represents fat which has been directly contributed by absorption from the intestine was shown by the failure to observe a significant increase in thoracic duct lymph lipid content in response to infusing a large volume of milky chyle intravenously. These results suggest that absorption of fat continues for more than 24 hours after its oral administration and is associated with a slow and prolonged delivery of fat by the stomach into the upper intestine. Stomach-fed dogs returned a significantly higher percentage of the fed fat in the lymph in 24 hours than did duodenum-fed dogs suggesting that the retention of fat in the stomach enhances capacity for effective lipid absorption from the intestine.

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Sterol Synthesis by Cells Cultured on Serum from Heat-Stressed Chickens.* (26915)

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Stress has frequently been linked to increased serum lipid levels as an etiologic factor(1,2). Experiments with hypophysectomized and adrenalectomized animals have indirectly incriminated stress hormones in hyperlipemia(3). Adrenaline and adrenocortical hormones have been shown not only to produce hyperlipemia *in vivo*(4) but also *in vitro* in cultures of rat adipose tissue(5,6,7,8). Similarly, corticotropin has been found to increase unesterified fatty acid levels *in vivo* (9). These reports are largely concerned with stress-hormone-induced fatty acid mobilization, however, mirroring the present increased interest in the role of unesterified fatty acids (UFA) in over-all animal lipid metabolism.

Bailey, Gey and Gey(10) found that MBIII lymphoblasts *in vitro* remove cholesterol esters from the nutrient medium, presumably by pinocytosis, and expel unesterified cholesterol into the medium. Azarnoff (11) has observed that organ cultures of the coronary artery and aorta of chickens, guinea pigs and rabbits convert C^{14} acetate to cholesterol, whereas this is not a characteristic of humans, dogs, cats and rats, although the latter do synthesize some type of digitonin precipitable steroid. Simms *et al.*(12) obtained a so-called "B-factor" or "lipfanogen" from human and animal sera (including chicken), which evidently causes lipid aggregation, resembling spontaneous atherosclerosis, in organ cultures of chicken and human arteries. In none of these instances, however, have these phenomena been linked to stress.

Recently(13) studies were conducted by us on the use of tissue culture techniques in biological tests for ascertaining stress in animals. Mouse fibroblasts cultured on a medium containing "stressed" serum (hereafter referred to as S-serum) produce a large number of small lipid droplets. This phenomenon is not elicited by "non-stressed" serum (hereafter referred to as NS-serum). It is demonstrable in rapidly growing, healthy cultures and is not a manifestation of degeneration.

The present report consists of results of tests made in an attempt to determine the nature of the lipid found in these cells after exposure to serum from stressed animals.

Materials and methods. The "Low line" strain of mouse fibroblasts (NCTC #2445) (14) was used for these tests. They were cultivated by inoculating 300 to 500 thousand cells per ml into either T-15 or T-60 flasks containing 2 or 10 ml, respectively, of a 10% horse serum 109 medium. After growth was well established (average cell numbers 1.5 million cells per ml), this medium was replaced, in half of the cultures, with a balanced salt solution (B.S.S.) containing up to 20% serum from stressed chickens and in the other half with serum from non-stressed chickens. Young White Leghorn chickens (age: 3-4 months) were subjected to heat stress for 24 hours at 102°F as described in a previous report(13). Total cell numbers in both control and test cultures were essentially identical at the end of the test period

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