THE HISTOLOGY OF FAT ABSORPTION WITH SPECIAL REFERENCE TO THE SITES OF ABSORPTION, THE MIGRATORY CELLS AND THE REGIONAL LYMPH NODES.

A Thesis submitted to the Faculty of Medicine in candidature for the Degree of Doctor of Philosophy.

by

ANN B. McNAUGHT.

The Institute of Physiology,
The University of Glasgow.

September, 1955.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

VOLUME ONE.

CONTENTS.

CONTENTS.

Volume One.

CONTENTS.

Volume Two.

INTRODUCTION.

INTRODUCTION.

Much has been written on the subject of fat absorption. In recent years this has been mainly on the experimental and biochemical aspects of the 'mechanisms' involved.

At the turn of the century many papers were published from the histological approach to the problem, but little recent work has appeared in this field. No comprehensive modern survey of the histology has been found, and there have been few studies on its comparative aspects. Undue distortion, resulting from the older histological methods of fixation and staining, gave rise often to conflicting reports and confusion in the interpretation of results. Many of the accompanying illustrations are difficult to correlate with the descriptive text, and naturally modern photomicrographic techniques were not available.

Accordingly, in the following pages, the historical/

historical setting is sketched, and an attempt made at critical restatement of the purely histological findings on fat absorption. Work is presented on the comparative aspects of the subject, with special reference to the sites of absorption, the nature and role of the migratory cells, and the regional lymph nodes.

Since these are all inevitably bound up with the conflicting theories on the 'mechanisms' and 'pathways' of absorption, an introductory chapter seeks to summarise the views held by leading experimentalists and biochemists who have worked in this field.

I. HISTORICAL REVIEW.

I. HISTORICAL REVIEW.

1. The 'Pathways' and 'Mechanisms' of Absorption - Experimental and Biochemical Approach.

A hundred years ago Claude Bernard (1877) published his pioneer observations on intestinal absorption of fat. He demonstrated the fine emulsification of fat in the intestinal lumen, the dependence of this emulsification on the presence of bile and pancreatic juice, the occurrence of milky lacteals and lymphatics, and a systemic hyperlipaemia at the height of fat absorption.

Towards the end of the last century, Immanuel Munk (1880-1900) did much experimental work in this field, his most notable opportunities being afforded by a girl with a thoracic duct fistula, from which he was able to recover, as neutral fat, 60 per cent of the fat ingested.

In the years that have followed these initial publications, controversy has raged on the exact nature of the factors involved and their relative importance. Since fat is insoluble in water, there has/

has been constant questioning as to the form in which it passes the epithelium of the mucosa, its fate within the epithelial cell, and the pathway of its further transport.

In the latter half of the nineteenth century it was generally thought that fat was absorbed as a fine oil-in-water emulsion. (Brucke, 1851; Munk,1900).

Heidenhain (1888) and Friedenthal (1900) introduced the idea that it might be dissolved in the cell lipids, after splitting into fatty acids and glycerol - the glycerol being absorbed by its solubility in water.

Subsequent experiments led many workers in the early years of the present century to emphasise the importance of lipolysis as a necessary step in fat absorption. Under the patronage of Pflüger (1900) this view gained increasing support, and was finally championed in its most complete form by Verzár and McDougall in 1936. These writers supported the conception that fat absorption was dependent on complete hydrolysis of glycerides in the lumen of the intestine. Pflüger (1900) suggested that fat, split by lipase in the presence of bile, the resulting fatty acids having /

having been saponified, was absorbed in the form of water-soluble, sodium soaps. Verzár, in view of the prevailing acidity of the upper intestine, and the demonstrable instability of alkali soaps at this pH range, himself put forward the view that after lipolysis, the non-diffusible, higher fatty acids, in water-soluble and diffusible combination with the paired bile acids, were able to enter the epithelial cell as a hydrotropic compound and, after dissociation from the complex, were resynthesised to neutral fat. The bile acids thus freed were adsorbed to the surface of the epithelial cells, and were again made available for the exercise of their hydrotropic function.

Since the fat in the thoracic duct lymph was mainly neutral fat, it had been tacitly assumed that the resynthesis took place in the epithelial cell, and was simply a reversal of the hydrolysis known to take place in the lumen of the intestine under the influence of the enzyme, lipase.

Sinclair (1929), however, pointed out that although some authors (Ewald, 1883; Hamburger, 1900) had claimed positive results, in vitro attempts to demonstrate/

demonstrate such synthesis of fat from its split products, in the presence of macerated mucosa, had been unsuccessful or inconclusive (Moore, 1903; Frank & Ritter, 1906). His own experimental work led him to believe that the absorbed fatty acids were transformed into phospholipids within the intestinal mucosa as an essential intermediary step in the resynthesis of neutral fat.

Verzár accepted this view and later incorporated it in his monograph.

The histochemical studies of Lison (1936), and the experiments of Artom and others (1935,1937), Sinclair (1936), Perlman, Ruben & Chaikoff (1938), in which labelled phosphorus or fatty acids were traced in their passage through the intestinal wall, gave ample corroboration that phospholipids were indeed formed in the epithelial cell.

It had previously been well established that neutral fat could be demonstrated, histologically and chemically, in the cells of the epithelium (Noll, 1910), and analysis of the chyle recovered from mesenteric lymphatics showed that almost all of the/

the contained fatty material was present as triglycerides (Moore, 1903).

Although an increase of lipoid material could sometimes be demonstrated in the portal blood during absorption of fat, there was sufficient reliable evidence to indicate that no significant increase of fatty material occurred in the portal circulation during absorption of neutral fat. (Zucker, 1920; Winter & Crandall, 1941; Little & Robinson, 1941).

Stressing triglyceride hydrolysis and resynthesis as they did, it followed that Verzár and his supporters held to almost total absorption by the lymphatic pathway into the systemic blood, and allowed that only negligible amounts reached the portal circulation by absorption to the capillaries.

The premise that fat must be hydrolysed before absorption found objection in the work of Mellanby (1927), who had shown to his own satisfaction that in the absence of lipase from the succus entericus, absorption of fat occurred in the cat intestine from which he believed lipase and lipolysis were thus significantly absent.

Finally,/

Finally, on the basis of a number of experimental findings which could not be reconciled with the lipolytic theory of the mechanism of fat absorption, Frazer (1938) suggested the Partition hypothesis, which postulated a partition of absorbed fat,(possibly predetermined by lipolysis), the fatty acid fraction (perhaps in solution due to the hydrotropic action of the bile salts) passing to the liver, and the glyceride fraction (absorbed as finely dispersed particles of unhydrolysed fat) by the lymphatic pathway to the systemic blood and the fat depots.

Since it seemed obvious that neutral fat could pass from the intestinal cell to the lacteal, and permeate many cell membranes in the body without question of preliminary hydrolysis, he found no difficulty in accepting that it might also pass the free border of the cell without initial breakdown.

Frazer further suggested the possibility that not all the fatty acid was destined for the liver, as some of it formed phospholipin in the intestinal cell and might travel by either route.

An increasing number of observations, chiefly those of Frazer and his colleagues, furnished added support for this view (1938-1950).

It/

It will be seen that there was general agreement on the main changes which fat might undergo during absorption. The occurrence of emulsification, hydrolysis, phosphorylation, and the importance of the bile salts, were recognised by both schools of thought. Their differences lay in the way these changes were brought about, and on their relative importance in the absorption of fat.

It was essential to Verzár's (1936) and Bloor's (1943) hypothesis that emulsification, by providing a larger, accessible surface area for the action of lipase, served only to facilitate complete hydrolysis; Frazer and his colleagues (1933-1950), on the other hand, thought that hydrolysis was partial and provided fatty acid, di- and mono-glyceride, as components together with bile salts, of the only emulsifying system found by them to be effective in the physiological pH range prevailing in the small intestine (Frazer & Sammons, 1945; Frazer, Schulmann & Stewart, 1944). This was also in contradistinction to Pflüger's suggestion that bile alone was effective, and to Mellanby (1927) who thought that acid soaps were probably the responsible emulsifying agents. Frazer showed that none of these provided stable, fine dispersion/

dispersion in the physiological pH range.

Far from recognising phosphorylation as an intermediary stage in glyceride resynthesis, Frazer regarded phospholipid in the intestinal cell as an end product in itself. While indicating that it might be a convenient, transportable form of fat, more suitable than triglyceride for many metabolic reactions, he published results strongly suggesting that phosphorylation represented an essential change in the interfacial structure of fat particles, needed to maintain normal stability and dispersion in the blood stream.

Zilversmit, Chaikoff & Entenman (1948) found that fat absorption did not stimulate the rate of phospholipid turnover in the mucosa, and this tended to substantiate the view that phospholipids were not obligatory intermediates participating in fat transport across the intestinal wall.

Barnes, Miller & Burr (1941), impressed by the fact that at the height of fat absorption, when neutral fat in the mucosa contained large amounts of the administered labelled fatty acid, only small amounts appeared in the phospholipid, lent support to this view.

The/

The part played by the adrenal cortex was also the subject of conflicting opinion. Verzár & Laszt (1935) regarded it as essential for normal phosphorylation in the intestinal cell, while Frazer (1946) thought its importance lay in its control of normal electrolyte balance, which was closely related to the absorption of charged particles. His own experiments (1947,1949) had suggested that glycerides passed through as a finely dispersed emulsion of negatively charged particles less than 0.5μ in diameter.

With regard to the bile salts, Verzár stressed their hydrotropic action, while Frazer, though accepting this, placed greater emphasis on their ability to produce fine dispersion of fat in the presence of stabilisers.

After the publication of Frazer's initial reassessment, the pendulum of current opinion swung from Verzár's to his interpretation of the evidence on fat absorption.

Experimental work by Farrarger & Collett (1950) confirmed that complete hydrolysis of triglycerides was/

was not necessary for absorption.

Sherlock and Walshe (1946) using a portal anastomotic vein for absorption studies in man, had inconclusive results with regard to absorption of fat. A portal lipaemia did seem to result, however, when lipase and fat were given together.

Auld and Needham (1951), in observations afforded by a patient with a traumatic chylothorax, found that, when stained neutral fat was given, a substantial amount of the dye was recovered from the chylothorax; whereas, when equivalent amounts of stained fatty acid and glycerol were given, little or no dye was recovered. These observations tended to corroborate Frazer's statement that fatty acids went via the portal blood stream to the liver, unhydrolysed glycerides via the lymphatics to the systemic blood stream and the fat depots.

Certain substances, for example 'Tween 80' (polyoxyethylene sorbitan monooleate) developed commercially as emulsifying agents, were used by various workers (Jones, Culver, Drummey & Ryan, 1948; Althausen, 1950) to produce a more homogeneous, finer emulsification of dietary fats. The addition of/

of such emulsifying agents to the diet apparently reduced the size of the fat globule and by this better dispersal of lipoid substances presented to the intestinal mucosa gave an increased rate and amount of absorption of fat and fat-soluble substances.

Becker, Meyer & Necheles (1950) found alterations in the chylomicron counts in the blood following the administration of this substance (Tween 80) with the fat. Such observations again seemed to lend support to the view that fat could be absorbed in the unhydrolysed state.

Reports by Brien and others (1952) that ingested Tween 80 seemed to hasten absorption of fat in normal persons were, however, followed by those of Tidwell & Nagler (1952,1953) that Tween 60, Span 60, etc., fed to rats and human subjects, led to no alteration in the absorption of fat as judged by the chylomicron counts and faecal fat estimations. Recently, Minard (1953) in vitro experiments, has found Tweens, 20, 60 and 80 to be hydrolysed by pancreatic lipase, thus by substrate competition, retarding the hydrolysis of corn oil, the whole effect being reversed by the addition/

addition of bile salts.

Recently, then, a new trend is detected in the literature. Webb, writing in 1952, pointed out that "a considerable body of evidence has been accumulating to cast doubt on the accuracy of Frazer's hypothesis."

Tidwell (1950), making a study of fat absorption in the rat, found lipaemias of equal intensity, whether neutral fat, or the free fatty acids prepared from it, were fed to the animals.

Reiser & Bryson (1951) found free fatty acids and triglycerides absorbed by the same route.

Bloom, Chaikoff and their colleagues (1950), fed palmitic acid labelled with radioactive carbon, to unanaesthetised rats, into whose thoracic ducts or lacteals, cannulae had been introduced. From 70 to 92 per cent of the absorbed labelled fatty acid was recovered as such from the thoracic duct, and 69 to 84 per cent in those cases where intestinal lymph was analysed. It is again of significance that results were materially the same whether the compound was/

was fed as the fatty acid or the triglyceride.

These authors demonstrated (1951) that the rate and route of fatty acid absorption in the rat depended on the length of the carbon chain of the acids. Stearic acid (C 18) was absorbed much more slowly and to a greater extent via the lymph system than were the shorter - chained members, such as lauric and decenoic (C 14 and C 10) acids, which, besides being absorbed more rapidly, apparently took routes other than the lymph, since only small amounts of them were found in it.

As a result of this and further work, they concluded (1951) that the transport of long chain fatty acids, whether of even or odd numbers of carbon atoms, was the concern almost exclusively of the lymph - a conclusion with which Frazer (1951) now agreed, pointing out (1952) that there was no essential incompatibility between the new interpretations and the Partition hypothesis, since the difficulty of absorbing long-chain saturated fatty acids by any route either than by the lymphatic pathway had long been appreciated by his school, and had been regarded as/

as one of the reasons for the selective rejection of such fatty acids in the sprue syndrome (1947,1949).

In his view (1951) partial hydrolysis in the ~~grit~~ *gut* lumen resulted in mono - and di - glycerides and fatty acids, which in the presence of bile, led to a fine emulsification of the residual glycerides.

Such emulsified triglycerides, together with long-chain fatty acids, penetrated the canals of the striated border of the intestinal epithelium, in particular absorption, and eventually passed, by the lymphatic pathway, to the systemic blood.

Mono - and di - glycerides were transformed into phospholipids, and eventually triglycerides, within the cell.

Short-chain fatty acids, and perhaps some phosphilipids, absorbed in the aqueous phase, passed into the capillaries of the portal stream.

Bergström, Borgström & Rottenberg (1950-1954) also studied absorption and distribution, in the rat, of labelled stearic acid, fed either incorporated in corn oil (by transesterification), or mixed with free fatty/

fatty acid from the same source. The route of absorption was found to be the same in either case. The labelled stearic acid was rapidly incorporated into the intestinal phospholipids. Lymph glycerides were found to contain about 90 per cent of the labelled stearic acid fed, but the specific activity of the plasma glycerides remained low, indicating rapid uptake of the labelled lymph glycerides by the tissues.

When labelled stearic acid was fed with corn oil glyceride or cholesterol, distribution of labelled acid between the glycerides, phospholipids and cholesterol-ester fatty acids was the same, irrespective of the form in which the active stearic acid was fed (i.e. whether fed as free acid dissolved in corn oil, transesterified with corn oil, or as cholesterol ester dissolved in corn oil).

Gregory pointed out (1954) that these results may be interpreted in one of two ways - that fat is completely hydrolysed in the lumen of the gut before absorption, and taken up as fatty acids, cholesterol and glycerol, being then synthesised to glycerides, cholesterol esters, and phospholipides; or that it is incompletely hydrolysed and absorbed partly in particulate/

particulate form, in which case it must be completely transesterified in the intestinal lumen or in the cells.

Borgström (1952) did show that during the pancreatic lipolysis of glycerides in the intestinal lumen of rats, a partial resynthesis of glycerides occurred simultaneously with hydrolysis. The fatty acids liberated by lipolysis were preferentially absorbed. Diversion of bile and pancreatic juice from the intestine almost completely abolished lipolysis and resynthesis of lipids.

Reiser and his co-workers (1952) raised further complications when they found that, though 25 to 45 per cent of labelled trilinolein fed to rats was hydrolysed to its constituent parts during absorption, the glycerol so formed was not reused in the synthesis of glycerides found in the lymph. The occurrence of the labelled carbon dioxide expired, indicated that some was quickly metabolised. Most of the triglyceride which had not been completely hydrolysed, and which appeared in the lymph, had apparently been hydrolysed to the monoglyceride, absorbed as such, and then resynthesised to triglyceride.

Reiser & William's later work (1953) on fatty acids suggested that, during their absorption, the formation of fatty acids - dihydroxyacetone esters, with subsequent reduction and esterification, might be a normal method of triglyceride synthesis. They showed that fatty acid esters of dihydroxyacetone were converted to triglycerides during absorption and only triglycerides were found in the lymph.

These results are quoted since they appear to throw fresh light on the mechanism of fat absorption, and to explain, in part, the recurring, contradictory findings and interpretations of the older writers. In this connection, it is to be remembered, as Frazer himself pointed out (1940), that much of the confusion may have been due "to the administration, under grossly artificial conditions, of large amounts of abnormal fats, and to interference with normal intestinal function and equilibria." There is also the more obvious reason, perhaps, that some of the work was done on herbivores, whose fat intake is normally low, and whose mechanism of absorption may, for that reason, differ from that in carnivores or omnivores with essentially different dietary habits and needs.

The/

The questions impeding complete understanding of the process of fat absorption have thus not been finally settled, and the problem awaits still further experimental and biochemical elucidation.

2. The 'Pathways' and 'Mechanism' of Absorption - Histological Approach.

i) 'PATHWAYS'.

(a) The Epithelium:

The foundation for histological work on absorption was laid by men like Henle (1838), who showed the intestine to be covered by a layer of epithelial cells. The free border of these cells in vertebrates was first depicted by the same writer in 1841. His drawing made it appear structureless and showed a cell membrane surrounding the rest of the cell almost as thick as the free border - an error attributable to the diffraction halo effect caused by the low numerical aperture of the objectives available at that time.

Goodsir (1842) was the first to notice that these epithelial cells apparently contained fat-like granules during fat absorption. He did not lay any stress on this fact, but imagined that "vesicles" close to the membrane had the property of absorbing, and that the epithelium itself was cast off prior to absorption.

Some/

Some years later, Perewoznikoff (1876) observed similar granules after feeding fatty acids and glycerol.

Gruby & Delafond (1843) saw what they took to be vibratile bodies on the free border of the epithelium covering the intestinal villi of the dog, and put forward the Cilium or Rod Theory suggesting that their function was displacement of chyle where it came in touch with the surface epithelium. It is probable, but by no means certain, that they did in fact see the striations which later gave rise to so much discussion.

These authors also found "chyle" in the epithelial cells which they regarded as organs specially charged to receive "raw chyle" and convert it to a "homogeneous chyle" for onward transmission.

They described the epithelial cell as having a cavity, with an external opening which sometimes gaped and at other times was tightly closed. In this respect, as with later authors, they failed to understand the true nature of the goblet cells.

Letzerich/

Letzerich (1866) described them as "vacuoles," connected by a system of canals with the central lacteal, and believed them to be the sole absorbents of fat. It was left to Eimer (1869) to point out that these "vacuoles" were in fact specialised mucus secreting cells.

It is interesting to note that,from then on, the histological study of fat absorption went hand in hand with that of the structure of the villus and its covering epithelium. Each series of observations gave rise to fresh speculation on the manner and site of absorption.

With the introduction of special dyestuffs it became possible to study the passage of fat through the mucosa. Brucke (1851) was probably the first to make use of this method, but thought that the epithelial cells were open at both ends and that the fat, after encountering a plug of mucus at the entrance to the cell, passed through as a fine emulsion. This view was upheld by Moleschott & Marfels (1854), but later denied by Donders (1856).

It was 1864 before Donitz introduced the idea of a "basement membrane." He asserted that the epithelium/

epithelium was separated from the parenchyma of the villus by a glass-like membrane "containing no visible pores."

Erdmann (1867) also held this view, and described the membrane as "sending processes around the epithelial cells" (as a cement substance), and into the stroma of the villus (as connective tissue trabeculae). He thought that fat travelled by the cement substance between the epithelial cells.

This latter theory stayed in vogue for some time and found support in the writings of Eimer (1869) & Watney (1876).

As late as 1902 Reuter, Kischensky, and others were still describing the passage of fat droplets between the cells, although Bruch (1853) had quite convincingly shown that fat entered the epithelial cells as little drops which united to form larger ones.

The nature of the free border had not yet been determined, and Funke (1855), in contrast to Gruby & Delafond's 'Cilium Theory', introduced his own "Pore Theory' to explain its structure. Having studied/

studied the rabbit intestinal epithelium, he interpreted the characteristic picture as being due, not to cilia, but to the presence of striations. He thought that these striations might represent "porenkanälchen," through which fat droplets might pass in absorption. He viewed the cells in side view and also in surface view, in which he described the striations as pores.

Kölliker (1856) independently demonstrated a striated border to these cells, and imagined them to be fine pores.

This view that the striations were in fact pores, found acceptance for some time, and Leydig (1866), in the French edition of his histological textbook, affirmed that the cuticle of the intestinal epithelium of vertebrates was traversed by small channels, which in surface view appeared to be punctate openings.

Hair-like processes on the epithelial cells of the frog intestine were noted by Thanhoffer (1874). These he thought showed lively movements and aided in fat absorption.

In/

In the vertebrates, the epithelial cells from the crypts of the colon in man were studied by Zimmermann (1898), who further complicated the conception of the free border by introducing the idea that an inner part had rods embedded in it, while the outer part was formed by fine "pseudopodia," each a continuation of an embedded rod.

Influenced by this work, Heidenhain (1899) went on to investigate the structure of the gut epithelium in salamander larvae. He too described a surface membrane composed of basal and superficial parts, both striated, but the basal part consisting of strong rods, the superficial part of fine protoplasmic filaments resembling cilia.

Prenant (1899) gave strong support to this view, maintaining that the membrane consisted of cilia which were fused together by a cementing substance.

For a time after this the pore theory, which had initially grown up round the idea that fats were absorbed as minute globules, fell into disrepute, although Heidenhain himself wrote equivocally about it (1907).

While the hydrolysis theory of fat absorption held sway, the rod theory gained currency, and both the pore theory and the physiological need for it lapsed.

Later work showed that here again, as in so many of the contradictory reports on fat absorption, confusion had arisen due to generalisations made from observations restricted to the study of one species.

Gage & Gage (1890) demonstrated that in the larvae of many amphibia, part of the intestine was genuinely ciliated. These cilia were lost before metamorphosis. This appearance was confirmed by Kaywin (1936). Newell & Baxter (1936) also made a careful study of the free border of the epithelial cells of the alimentary canal of some invertebrates. They found sometimes a true ciliation, sometimes what they called a "rod-border," and sometimes (in mid-gut of insects) a "brush-border." Histological examination of the membrane was finally presented in its most complete form by Baker (1944).

From the study of intestinal cells, again mainly of amphibia, he submitted convincing evidence that the/

the brush-border contained a system of fine canals, running at right angles to the surface, through which, particles rather less than 0.5 μ in diameter might pass. The constancy of his observations, in longitudinal and transverse section, and in preparations using different methods, made it unlikely that the canals were artefacts. His demonstration of pores coincided with the reintroduction of the idea of particulate absorption, and provided the sort of structural basis which Frazer's physiological findings required. The hypothesis that fat could be absorbed as such, gained weight from the fact that two entirely different and independent lines of investigation pointed in the same direction.

Wotten & Zwemer (1939) claimed to demonstrate fat actually passing through these canals. They published photographs which showed large drops in process of absorption. These were described as having an hour-glass appearance, one bulb lying in the gut lumen, connected to the other, within the cell, by a hair-like constriction passing between the striations of the cuticular border.

Baker in a later investigation (1951), using

Sudan/

Sudan Black on very thin sections (6μ) of the intestinal epithelium of the mouse during the absorption of fat, found spindle-shaped particles of lipoid material within the free border of the cells. These spindles corresponded in position with the canals previously described. A convincing series of photomicrographs substantiated this claim.

Although it had long been recognised that the cells of the lining epithelium of the gut became filled with fat globules during absorption, Jeker (1936) was the first to present a detailed histological picture. In his study on normal rats, droplets were maximal in size and number about six hours after ingestion of fat, and gradually disappeared from the cells during the course of the next few hours. After physiological fast there were no such droplets to be seen, but they made their reappearance within twenty minutes of the start of a meal. Droplets present at the peak of absorption were graded in size from large drops situated above the nucleus to small ones in the basal part of the cell.

Absorption did not start in all parts at the same time. Some villi showed absorption, while neighbouring/

neighbouring villi contained no sudanophilic material at all. Similarly, individual villi in the initial stages, showed the stain only in those cells situated at their tips.

Certain differences were seen in the intestinal epithelial cells of the frog (Leach, 1938). Here the cells became elongated during absorption of fat, and the maximum deposit was present usually twelve hours after feeding. The size of drops was much smaller in animals kept at low temperatures, and a few fat droplets were still found even at the end of a fast lasting twenty-eight days.

Recently, Volk & Popper (1950) with the aid of the fluorescent microscope, demonstrated fine fat droplets in the intestinal epithelial cells of the rat, especially in the upper part of the jejunum.

b). <u>The Villus</u>:

The absorption and further transport of fat cannot be properly understood without a knowledge of the structures and stroma of the villus.

Many of the earlier workers wrote at length on the/

the nature of the substance separating or connecting the epithelium with the stroma of the villi. Some of them supposed that the epithelium of the frog's intestine had an intimate connection with what were called "connective tissue corpuscles," or branched cells of the parenchyma. Eimer (1869) gave drawings of these, and demonstrated fat particles in the processes of the branched cells. Similar assertions were made for mammals. This writer envisaged a system of fine channels connecting the central lymphatic with the epithelial cells.

Such connections were denied by other writers, and, as has been shown already, Donitz (1864), Erdmann (1867), & Debove (1874), eventually demonstrated the presence of an unbroken membrane separating the epithelium from the stroma of the villus.

As Watney (1876) pointed out, this question of the existence of a basement membrane had important repercussions on the theories of fat absorption.

The older writers believed that absorption took place directly through openings on the surface of the gut. Asellius (1628) ascribed open mouths to/

to the "chyle vessels" of the intestine. Lieberkühn (1745) & Cruikshank (1790) believed that the central canal of the villus opened on to the surface.

Others, after examining the villi of many animals, denied the existence of openings on the surface, and thought that the "chyle-vessels" had their origin in a fine network of lymph-capillaries within the villi themselves. (Krause, 1837; Goodsir, 1842; Weber, 1847; Nuhn, 1849.)

Fohman (1827) injected quicksilver to the lymphatics of fish and found that none escaped into the intestine. Although numerous writers now thought of the lacteals as blind-ending, few had any theories on how fat traversed the villus to reach them.

Lacouchie (1843) seems to have been the first to report the movements of the villi, and to have had the conception of the "aspirantes et foulantes" pump mechanism attributable to a system of muscles. Gruby & Delafond, in the same year, independently discovered these movements. Brücke (1851) confirmed that they were due to bands of longitudinally arranged muscle fibres which ran to the tips of the villi, and were processes from the muscularis mucosae;

Hewson/

Hewson (1846) considered it probable that the villi were thus erected "in order to make the small absorbents stand rigidly open" and so convey chyle to the first pair of valves.

The idea that the granules of fat pressed gradually through the substance of the villus, not in definite preformed ways, but among the parenchyma, was introduced by *Frerichs* (1846), and upheld by Virchow (1853), Funke (1855), Donders (1856) & Donitz (1864).

Heidenhain (1858), however, put forward the very definite theory (based on histological findings which were often inadequate and inconclusive) that there was a direct transition, via the branched processes of the parenchyma, from epithelial cells to chyle vessels, which, he held, were the preformed ways traversed by fat.

Teichman (1861) by injection techniques, again satisfactorily demonstrated that the lacteals of the villi were closed, but was contradicted in the following year by von Recklinghausen, whose injection material left the vessel and entered the stroma of the villus. He, however, demonstrated that the lacteal/

lacteal was lined by an endothelium. His (1863) confirmed this latter observation, but upheld Teichman regarding the injection.

The matter was not allowed to rest there. In spite of these findings, Fles, in 1865, & Zawarykin (1869) were still contesting the presence of a membrane lining the upper part of the central lymphatic of the villus, and they were supported in this by the work of Basch (1865), whose injection to the lymphatics not only filled the central canal but penetrated the stroma of the villus in a regular network of lines which he took to be preformed pathways for the passage of absorbed fat.

Kölliker (1856) while holding to the former opinion that the lacteal was blind-ending, summed up the controversies by saying that the paths taken by fat in the tissue of the villus were still unknown to anatomists.

Watney (1876) finally described the central canal as club-shaped, its walls composed of endothelial "plates," and the stroma of the villus as reticular tissue containing lymphocytes and other free cells. Through this soft, semifluid mass, particles of fat could be pressed by the contraction of the muscle tissue surrounding/

surrounding and emptying the chyle vessel. This, with subsequent erection of the villi, served to promote absorption.

Grunhagen & Krohn (1889), and Reuter (1902), emphasised this growing conception that the fat passed from the epithelial cells into the subepithelial spaces in the connective tissue of the villus - a view with which Heidenhain later agreed (1891).

Jeker (1936), and Wotten & Zwemer (1939) described the fat content of the columnar cells being discharged into the interstitial spaces of the core of the villus, and finally draining into the central lacteal, which was squeezed by the contraction of the villus, and its lymph conveyed to the submucosal lymphatic plexus. These latter authors also described the passage of fat globules directly into the marginal blood capillaries of the villus. Their illustrations are by no means clear, and it may be that the thickness of their frozen sections (10 - 40 μ) accounted for this anomaly.

The fluorescent microscope, while confirming the entrance of fat droplets to the lacteals, failed to/

to reveal their presence in capillaries (Volk & Popper, 1950).

One other phenomenon had been noted. In 1861, Rindfleisch found round elements among the deeper layers of the epithelial cells. Eberth (1864) considered that some of these had wandered in from the mucosa. These cells were noted subsequently between epithelial cells and within the stroma of the villus, by many of the older writers, and were described as "lymph corpuscles" by Watney (1876).

Schäfer (1912), seeking to explain the transference of fat from the cells of the epithelium to the lumen of the central lacteal, and noting the presence of fat-laden leucocytes within the stroma of the villi, attributed this function to them. He compared them in this respect to the amoeboid cells of the lungs, which transfer carbon particles from the alveoli to the regional lymphatics. The suspected nature and function of these migratory cells, together with the role of the regional lymph nodes, is discussed in fuller detail in subsequent chapters.

In the frog, where absorption apparently proceeded/

ceeded more slowly than in mammals, Schäfer stated that no fat was to be found anywhere but in the epithelium, in the lacteals and in these leucocytes. In the guinea-pig he described many such phagocytic cells and believed that they took up all of the absorbed fat after it had passed through the epithelium.

He, however, found that puppies fed on milk showed streaks of fatty material extending from the epithelium to the borders of the lacteal, without the intervention of the so-called phagocytic cells. It thus appeared that absorption could take place so rapidly that the absorbed fat, after being finely divided in the epithelial cells, might be set free within the stroma without being taken up by these cells.

c) The Lymph Vessels and Further Transport:

With regard to the lymph vessels of the small intestine, Schäfer (1912) pointed out that these were arranged in four networks, one in the mucosa, one in the submucosa, one between the two layers of the muscular coat, and the fourth in the serous coat. The vessels forming the network of the mucosa appeared to/

to be mainly or wholly destitute of valves. They received the lymph from lacteals of the villi, and were in communication with the network of the larger, valved vessels of the submucosa. Schäfer drew attention to their very free communication, especially in the neighbourhood of the lymph - nodules which broke through the muscularis mucosae, and which were almost surrounded by sinus-like lymph-vessels. From this submucosal network, efferent valved vessels passed through the muscular layers, receiving lymph from the intramuscular network and entering the subserous network. This conveyed lymph to the mesenteric lymphatics, and root of the mesentery lymph node.

Since Asellius (1628) first described the lacteals full of chyle, a great deal of attention has been paid to the part played by these vessels in absorption.

There is no doubt about the presence of fat in the lymph of some of them during active fat absorption, and sudanophilic material has been demonstrated in lacteals, submucosal and other vessels of the intestinal network by several histologists, including Jeker/

Jeker (1936), who found that the central lacteals in the intestinal villi of the rat showed a faint yellow dust 10 - 20 minutes after ingestion of fat. Thirty minutes later these droplets were seen in the lymphatic vessels as far as the submucosa. Fischler positive material was similar in distribution and amount.

At 1 - 2 hours this material, which was regarded as fatty acid, had increased within the stroma and lacteals and seemed to indicate that some at least of the fatty acid (or intermediate phospholipid) had escaped transformation back to neutral fat.

At the end of 6 hours, the serosa and mucosa of the intestine had a milky-white colour, and larger white lymphatics could be seen in the mesentery.

With the Sudan dye he used, large fat droplets could be seen in the central lacteal, often uniting into a homogeneous red mass. Very little Fischler positive material was seen at this stage.

Verzar & Frazer both believed that the wall of the lymphatic vessel was not so impermeable that a fine emulsion of fat could not pass through it, without/

without postulating the intervention of phagocytic or amoeboid cells.

Volk & Popper (1950) demonstrated fat droplets in the lymphatics of the submucosa and muscularis one hour after the administration of corn oil. None were seen in the large subserous vessels at this time. These too, however, shared the fluorescence twenty-four hours after ingestion.

Striking histological proof that the bulk of absorbed fat entered the lymphatic system and was conveyed thus to the systemic blood, was provided by the introduction of chylomicron counts. Examination of the blood under dark ground illumination reveals large numbers of minute, actively moving specks of light. The presence of these small fat-laden particles in active Brownian movement was first noted by Edmunds (1877) & Neumann (1907). Gage & Fish (1924) studied the digestion, absorption and assimilation of fat in man and animals by chylomicron counts, noting the appearance of *chylomicrons* in blood and chyle ½ - 1½ hours after the eating of fatty foods. It took 6 - 10 hours for their subsequent disappearance.

More/

More recently, the method was used extensively by Frazer & Stewart (1937-8), who considered that the particles seen were essentially fat with an adsorbed protein film on their surface. Simultaneous estimations of the neutral fat, phospholipid, cholesterol, and protein in the blood showed that only the neutral fat-content varied in a manner parallel with the particle counts. These authors found the agreement between the neutral fat and the number of particles extremely close. An increase in particles was always found after a meal containing fat, but never after the ingestion of carbohydrate or protein alone.

In a group of experiments on rats, simultaneous samples of systemic and portal blood were examined at regular intervals following the introduction of fat or mixtures of fatty acid and glycerol in equivalent amounts into the small intestine. With neutral fat, a marked increase in particles occurred only in the systemic blood. Fatty acid and glycerol gave an increase in the portal blood. Systemic counts in human subjects served to corroborate these findings.

It was difficult to reconcile these results with/

with the supposition that all neutral fat must be hydrolysed before absorption.

In response to feeding rats on a diet to which Sudan-stained neutral fat had been added, Frazer (1938) found deep staining of the fat-depots throughout the body with only slight staining in the liver. Feeding with equivalent amounts of Sudan-stained fatty acid and glycerol produced practically no staining of the fat depots, but gave more marked staining of the liver. Suggestions that fat-soluble stains dissociated from fat during absorption in rats failed to explain the distribution in these experiments.

Taken with chylomicron counts, these findings strongly supported his conception that alternative pathways existed, and that, while fatty acid might pass to the liver by the portal circulation, neutral fat was destined for the systemic blood and the fat depots.

ii) "MECHANISM" -

(a) Form in which fat is absorbed:

Attempts were made at intervals to determine, by histological methods, not only the route, but the form in which fat entered the epithelium in absorption, and the changes it might undergo in its passage through the cell.

Krehl (1890) & Altmann (1894) believed they had demonstrated that fats were absorbed after splitting into fatty acids.

Hofbauer (1900) fed fat stained with alkanna (a red dyestuff believed by him to be soluble in fat but not in soaps), and found red fat droplets inside the epithelial cells during absorption. He concluded that the stained fat was absorbed as an emulsion, without being split up.

Pflüger carried out numerous studies (1900-1903) and strongly opposed this view. He pointed out that, since the colouring matter was soluble in bile, soaps, etc., its absorption did not necessarily indicate the passage of particulate fat.

Vital/

Vital staining was again employed in attempts to show whether or not all the fat was in fact split up before absorption. A series of controversial and inconclusive papers followed. (Friedenthal,1900; Hofbauer, 1901; Exner, 1901; Pflüger, 1901.)

Verzár, discussing these later (1936), stated that the alkanna, being soluble in lipoids, could have entered the epithelial cells with or without the fat, and if the fat was split up during absorption, and later resynthesised in the cell, the dye could have met and restained it there.

It seems, however, unlikely that the dyestuff would forsake its association with neutral fat to enter selectively the more complicated lipoids of the cell.

André & Favre (1906) saw the same histological pictures after feeding with soaps as after feeding with fat. This was interpreted to mean that at least some of the soap was broken down again to fatty acids and absorbed as such.

In 1909, a similar discussion arose in America between/

between Whitehead & Mendel.

Whitehead (1909) found that after absorption of a fat-emulsion stained with Sudan-red, the mesenteric lymphatics of rabbits were white, not red. This favoured the opinion that complete lipolysis took place before absorption.

This was immediately followed by Mendel's paper (1909), in which he stated that, on repeating the same experiment, the lymphatics were always stained red. He, however, added the rider that the fat could have met the dyestuff in the cells and been restained there. On this theory the lymphatics would be dyed, whether absorption took place as an emulsion, or as fatty acids and glycerol.

It is interesting to note in this connection that Verzár and his colleagues (1936) repeated the experiment on rabbits and found in confirmation of Mendel, that the lymphatics of the mesentery were always stained red after feeding Sudan-tinted milk, cream or olive oil. When they repeated the experiments on rats, the lymphatics of the mesentery were milky white, and only the interior of the gut at some places showed a dark red emulsion, and the epithelial cells/

cells in the upper parts of some of the villi were stained red. This result in the rat was taken as confirmation that fat was not absorbed as an emulsion, but underwent some type of breakdown in the gut which led to the separation of the dyestuff from the fat. The differences obtained in the two species was explained in terms of slower rates of absorption in the rat than in some races of rabbit.

Here again it would seem that obvious points had been overlooked. Amongst other things the relative lengths of the small and large intestines vary considerably, as must the part played by fat in the normal diet of each, and the length of exposure to lipolytic and emulsifying agents.

It became imperative to try to find histological methods of differentiation between the various fat substances.

In 1904, Fischler published his method which claimed to differentiate between neutral fat, and fatty acids and soaps. Rossi's method (1907) was a modification of this and similar techniques were employed by Lorrain-Smith & Mair (1908), Ciaccio (1910), and Lamb (1910).

These methods were severely criticised by Kaufmann & Lehman (1928), and in spite of repeated attempts, no successful histological method of differentiating triglycerides from fatty acids has yet been established.

Nevertheless, several important histological investigations were carried out, and interpreted, on the basis of such methods.

Amongst these were the experiments of Weiner (1928) and Jéker (1936), whose observations at Verzár's instigation, provided the most intimate histological picture of events during fat absorption.

In this work, interpreted in favour of the lipolytic-resynthesis theory, Sudan gave minute yellow points of colour in some of the cells and lacteals in the early stages of absorption. These, together with a positive Fischler reaction, were regarded as due to absorbed fatty acids.

Over the first half hour, Fischler positive material increased in cells, stroma and lacteals, and was present in both supra - and infra-nuclear positions in the cell.

After/

After 1 to 2 hours of absorption this material progressively diminished, while distinctly sudanophilic droplets made their appearance and increased continuously in size and number till a maximum was reached after about 6 hours.

These appearances were interpreted to mean that fat was absorbed as its split products, and that resynthesis of neutral fat started and became progressive after 1 - 2 hours, only a little fatty acid or lipid (perhaps the 'intermediate phospholipids') escaping to the chyle.

In rats in which absorption of fat was inhibited by monoiodoacetic acid, the histological picture was quite different. With Sudan, practically no fat could be seen in the mucosa after 6 hours. Where present, sudanophilic material was also positive with Fischler's stain. Although not so complete, a similar inhibiting effect was seen when phlorrhizin was injected. This picture resembled most closely that obtained after 20-30 minutes of normal absorption, and was regarded by Verzár (1936) as evidence that fatty acids, in combination with bile acids, had diffused into the epithelial cells, but that resynthesis/

resynthesis of neutral fat had been inhibited by monoiodoacetic acid, or phlorrhizin, presumably by their interference with the phosphorylation process within the cell.

The corresponding picture was interpreted differently by Frazer (1940), who pointed out that the cell damage caused by such dosage of these toxic substances could easily account for the failure in normal absorption.

The appearance presented in the normal rat was taken by Frazer as indicating particulate absorption of fat, the large drops forming, by simple coalescence, in the cell.

Frazer could see no reason why the neutral fat in the 6 hour specimen should necessarily have been derived from the fatty acid of the 30 minute section. Neutral fat, absorbed as such, would give an identical histological picture.

He (1943), therefore, repeated Jéker's original experiment, feeding neutral fat as before, and also feeding a mixture of glycerol and free fatty acid.

When he fed neutral fat and examined sections taken/

taken from rats killed less than 1 hour after feeding, he found the characteristic picture described by Verzár & Jéker as representing fatty acid absorption. This was present over a certain area of the small intestine; in regions far removed from the source of the pancreatic juice, he found the picture which Jéker described as occurring only after 6 hours of absorption, in which the cells were packed with large globules of neutral fat. Rats killed a short time later showed the "fatty acid" absorption picture only in a few inches of the duodenum immediately caudal to the ampulla of Vater. Above the ampulla there was no sign of fat absorption, and in the lower duodenum and in jejunum a picture similar to Jéker's six hour stage was found.

When mixtures of free fatty acid and glycerol were fed, he found the fatty acid absorption picture (a fine granular deposition, staining rather brown with Sudan III) over a wide area of the small intestine, but, what was more significant, this stage was not followed by one of deposition of neutral fat in the cells. The fatty acid appeared to be absorbed unchanged. Taken together, these findings strongly supported/

supported his conception that neutral fat was not absorbed after initial lipolysis.

Frazer and his colleagues also showed that the rate and amount of lipolysis in the intestine could be increased by adding an active preparation of lipase to the fat fed to rats. Under these conditions the histological picture resembled that obtained when fatty acid (oleic acid) was fed - the cells were filled with fine granules.

Small amounts of sodium cetyl sulphate added to the oil fed, prevents the hydrolysis of fat by lipase, and in rats so fed, the histological picture was like that when neutral fat was fed alone - intestinal cells were filled with large globules and lacteals were milky.

These results also pointed to lipolysis not being essential to the absorption of fat.

The traces of free fatty acid and monoglyceride found in the intestinal emulsion of neutral fat could give rise to the traces of Fischler positive substance in stroma and lacteals, attributed by Verzár to fatty acid escaping the process of resynthesis.

Frazer/

Frazer (1949) observed the presence of large fat droplets in intestinal cells of rats fed 1 c.c. of olive oil with 1 c.c. of water. Very little of this fat appeared to pass into the areolar tissue of the villi or into the lacteals.

The addition of choline chloride, in a concentration of 0.5%, resulted in the appearance of much fat in the areolar tissue, while the epithelial cells, in which the fat was more finely dispersed, were more rapidly cleared.

Tidwell (1950) achieving a similar effect with parenterally administered choline, suggested that this increased rate of absorption could not be exclusively the result of increased emulsification of fat in the intestine, but must involve choline in some other important role, such as the stimulation of phosphorylation.

Baker (1949,1951) in the cytological investigation referred to earlier, which was designed to discover whether fatty food was absorbed by the intestine of the mouse in particulate form, or in aqueous solution after hydrolysis, found fewer Sudanophilic particles within the free border after fatty acid/

acid feeding than when the food contained triglycerides, and those present were not so intensely coloured by Sudan Black. His experiment showed that fatty acid could be taken into the free border apparently in particulate form, but did not show that the sudanophilic particles seen in the free border after triglyceride feeding consisted of fatty acid.

Finally, evidence of quite a different type was brought to bear on the theories of the mechanism of fat absorption. At first this evidence was sought by experimental means, but its completion was achieved histologically.

As early as 1899, Constein had stated that lanoline was not absorbed.

Henriques & Hansen (1900) gave rats a mixture of fats and paraffin oil, in fine emulsion, and found that, while the fat was absorbed, the paraffin was excreted quantitatively. This seemed, for a time, to show that the emulsion of fat-like substances was not enough for their absorption, and lent support to the lipolytic theory.

Bradley/

Bradley & Gasser (1912), however, fed a dog an emulsified mixture of olive oil and liquid paraffin, and found both substances in the thoracic lymph in about the same relative proportions as in the emulsion fed. They suggested a mechanical absorption of droplets of hydrocarbon oil, but seemed to miss the implication that the olive oil might similarly have been "mechanically" absorbed in the unhydrolysed state.

These experiments, repeated by Bloor (1913), gave opposite results. He administered a liquid hydrocarbon mixture and also vaselin, alone, and in emulsions in olive and cocoanut oils, and recovered in the faeces 85 - 100% of the hydrocarbons administered.

He also gave similar mixtures to animals with thoracic fistulas, and prepared the unsaponifiable fraction from the chyle. Results seemed to indicate that, although these unsaponifiable substances could be readily emulsified in the small intestine, they remained unabsorbed.

In view of these contradictory findings, Gage & Fish in 1924 set out to test the digestibility of castor/

castor oil and liquid paraffin.

They found no increase in the chylomicrons, and interpreted this to mean that no absorption of either substance took place.

Mellanby (1927), in experiments similar to Bloor's, reported no absorption of petroleum oil in the intestine or mesenteric lymphatics following ingestion.

The results of Channon and his co-workers (1926-1934), however, established that absorption of hydrocarbon oils could and did occur.

Their initial experiments (1926) with the unsaturated hydrocarbon, squalene, showed that some was absorbed by the rat and appeared in the liver.

Cetyl alcohol and other allied substances were also found to be absorbed to some extent (1928).

Eventually (1929), by studying the unsaponifiable fractions of livers of rats and pigs which had received liquid paraffin in their diet, they found definite indications that this substance had been absorbed.

It/

It is significant that in the experiments yielding negative results, either the hydrocarbons were given in the crude, unemulsified form, or the degree of dispersion was not checked microscopically, and no guarantee of its fineness ensured.

Twort & Twort (1932-3) were the first to publish papers on the histological changes thought to be due to the absorption of these substances in mice, rabbits and rats.

After application of liquid paraffin to the skin, the livers were found to show periportal infiltration by small round cells, which became larger, and eventually underwent disintegration with formation of foamy cytoplasm, coarse vacuolation, and ultimate replacement by globules of oil, the nature of which was undetermined.

The authors believed that the oil had access to the internal organs from the alimentary tract after its ingestion by the animals from the skin.

Tantini (1935) fed liquid paraffin by mouth to guinea-pigs for a four-month period, and reported that/

that the oil could be demonstrated, histologically, in the hepatic cells.

These results, taken together, indicated, not only that fat-like substances might be absorbed from the intestinal tract, but, since these substances were unsaponifiable, that a mechanism of absorption existed other than that provided by lipolysis.

Stryker (1941) found absorption of liquid paraffin demonstrable histologically in human subjects, rats, guinea-pigs and rabbits. In the latter, intestinal lesions seemed to suggest that absorption had taken place in the jejunum, ileum, and first part of the colon.

These lesions took the form of small white nodules visible to the naked eye at post-mortem examination. Similar white nodules were seen on the surfaces of the enlarged mesenteric lymph nodes. An occasional nodule could be seen in the liver.

Histologically, aggregations of vacuolated macrophages were seen in the lamina propria, especially near the tips of the villi. Extracellular vacuoles,/

vacuoles, often bordered by giant cells, made their appearance in animals fed with the oil for prolonged periods. Sudan IV showed sudanophilic material at the site of these extracellular and intracellular vacuoles. The material, however, stained light orange in contrast to the orange-red colour of the adjacent adipose tissue, and showed no reduction with osmic acid. Similar intracellular and extracellular vacuoles were present in the mesenteric lymph nodes.

The extent of the changes increased with the length of time liquid paraffin had been fed.

In rats and guinea-pigs such lesions were absent in the intestinal wall, but mesenteric lymph nodes in all three species showed the typical 'oleophages', and later the extracellular vacuoles.

A close correlation existed between the microscopic observations and the results of the chemical analyses, which revealed that the oil present was of the hydrocarbon type.

Stryker, himself, seemed to accept the lipolytic theory of fat absorption, and apparently assumed that the absorption of the mineral oils was by a process/

process other than that which characterised the absorption of ordinary fats and oils.

It was left to Frazer (1942) to demonstrate conclusively, by histological and biochemical methods, that absorption of emulsified liquid paraffins undoubtedly occurred in the rat intestine.

Frozen sections, stained with Sudan, showed droplets of the order of 0.5μ diameter, in the cells of the intestinal epithelium. Control experiments with the unemulsified paraffin showed no Sudan-stained droplets in the cells.

By serial investigation at thirty minute intervals, the passage of the absorbed fatty material was observed to the systemic blood stream. Chylomicron counts, similar to those obtained with olive oil, showing a peak of two hundred particles per field, were obtained with good paraffin emulsions. Coarser emulsions gave a peak of one hundred particles per field, and unemulsified paraffin about twenty or thirty.

Biochemically, the absorption of well-emulsified paraffin was found to be as high as sixty per cent of/

of the quantity administered, which compared favourably with the absorption of olive oil in control animals over a similar time-period.

Sheshkes, Jeyer & Stare (1950) showed that fine emulsification improved paraffin absorption from rat intestinal loops by 23% and the absorption of corn oil by 26%.

The additional work of Bernard & Scheitlin(1952), in which mineral oil, fed in small amounts to rats, was traced by its characteristic absorption spectrum, left little doubt that unsaponifiable oils could be absorbed.

These results supplied some of the most convincing evidence in support of a theory of mechanical absorption of particulate fat, although Lundbaek & Maaløe(1947) were unable to confirm the absorption of paraffin emulsions said to be as low as 0.3 μ in particle size.

b) Intracellular Changes in fat during absorption:

Drage (1901) tried to give a histological analysis of the intracellular changes during fat absorption, and Noll (1908-1910), believed he had demonstrated/

demonstrated the intracellular synthesis of soaps and neutral fat from fatty acids. He thought that during fat absorption, neutral fat was thus laid down within the cell, whenever the rate of entry of fatty material into these cells was greater than the rate of removal.

The significance of Jéker's and Verzár's findings, and Frazer's interpretations, has already been discussed. In the case of the latter, only 'mechanical' coalescence of fat droplets was assumed to occur in the cell, while the former inferred that they had demonstrated, histologically, a resynthesis process.

Other aspects of the cell's activity during absorption were covered by Cramer & Ludford (1925), adherents of the lipolytic-resynthesis school, who studied the role of the Golgi apparatus in the specific functional activities of the cell.

In active absorption and assimilation of fat by the intestinal epithelium, they found that this structure swelled up and formed a network filling the supranuclear part of the cell. The globules of 'synthesised fat' lay in its meshes. In all cases the/

the extent of its alteration depended on the amount of fat absorption taking place. When this was impaired by fasting or deprivation of the necessary vitamins A and B, the change in structure was much less marked.

On the other hand the mitochondria remained unaffected by the processes of fat absorption, and the authors concluded that, in the synthesis of fat within the intestinal epithelium, the Golgi apparatus was the cell structure chiefly concerned. Weiner, (1928), combining several methods, including that of Fischler for fatty acids, carried out an extensive study of fat absorption in frogs, mice, rats and bats. He distinguished two stages of fat absorption. Sometimes he found "fatty acids" in the epithelial cells, in the villous, stroma, and lymphatics after 7½ minutes. This was followed by a "stage of deposition of fat," when fat droplets appeared in the cell over the nucleus in the Golgi apparatus. These small drops coalesced to form larger drops. His own impression was that at this stage the neutral fat was surrounded by a layer of fatty acid, and that the Golgi apparatus had, not so much a synthetic, but a "depository" activity in the cell. He described the mitochondria dividing/

dividing into fine granules during fat absorption and thought that they might be the "synthesising organs".

In summary, fat droplets are apparently absorbed through the pores of the striated-bordered epithelium of the small intestine, maximal absorption occurring, in those species examined, at the junction of the upper and middle thirds of the jejunum.

Villi show functional independence, and fatty inclusions are graded downwards in size from tip to root of villus and from luminal to basal aspect of the cell.

Fat droplets are discharged from the epithelial cell to the underlying areolar tissue, and, by means of the 'villus pump' mechanism, are pressed through the stroma of the villus and the endothelial lining of the blind-ending central lacteal, without the intermediate agency of the migratory cells, the role of which is still in doubt.

The Sudan-stained picture, together with chylomicron counts, strongly support Frazer's conception that, while fatty acids may pass in the portal circulation to the liver, neutral fat via the lymphatic route is destined for the systemic blood and/

and the fat depots.

Although no distinctive histological method exists for the accurate differentiation of intracellular fatty acid and neutral fat, careful microscopic examination of sections stained with the red Sudan dyes shows a subtle difference in colour reaction, shape and size of the cell inclusions in either case.

Such examination, together with the convincing evidence supplied by particulate absorption of emulsified hydrocarbon oils, lends added weight to Frazer's interpretation of events.

Both biochemical and histological work seems to indicate that both 'mechanical' and 'chemical' changes may take place in the intestinal cell, but without new developments in histochemistry, histological methods alone are unlikely to elucidate further the exact nature of these intracellular changes.

3. Effect of Diet, Species and Age on Fat Absorption.

Diet:

Large amounts of fat can be absorbed, but the absorption is slower than for other foodstuffs.

Rubner (1879) first indicated that, while the average diet of man might contain about 50 grams of fat per day, 100 to 150 grams could be absorbed without difficulty.

Pettenkofer & Voit (1873) showed that a dog of 30 kilograms was able to absorb 343 grams of fat, and Verzár & Macdougall (1936) found that rats of 200 grams absorbed 3.5 grams in one day.

More recently, Rekers, Abels & Rhoads (1943) pointed out that the quantity ingested by human subjects or dogs could be increased to 200 or 300 grams per day without any significant diminution in the percentage absorbed.

Fats of different composition are not all equally well absorbed (e.g. olive oil, lard, plant fats are absorbed up to 98%; sperm oil only to 15% - Verzár, 1936).

Verzár interpreted these differences in absorption/

tion as due to the different degrees to which fats are emulsified and split up by lipase.

A surplus of neutral fat, of course, results in delayed emptying of the stomach. Normally it is impossible for more than carefully regulated quantities of fat to pass into the small intestine at one time (Frazer, 1940). The speed with which they leave the stomach is determined largely by their melting point and viscosity.

Wotten & Zwemer (1939) found that smaller quantities, and lower melting point fats, were in general absorbed more rapidly. With higher melting points and larger quantities, absorption proceeded at a slower rate, but the histological picture was similar. Beef fat appeared to be more finely divided than cod liver oil. They thought that the lower viscosity of the oil might enable the cells to absorb larger drops. Their observations suggested that there was little difference in the absorption of high or low melting point fats of plant or animal origin.

In the past thirty years several important histological investigations have been carried out to determine the/

the effect of vitamin deficiency on the absorption of fat.

Mottram, Cramer & Drew (1922) studied the question in rats, which after a twenty hours' fast, were artificially fed with one of three diets. Controls were given the standard, vitamin-free diet of starch, casein and salt mixture, to which was added olive oil. The histological picture of the osmic stained epithelium of the small intestine showed large globules of fat between the nucleus and the free border of the cells. There were very few droplets in the reticular tissue of the villus and where present these were relatively large.

A second group of animals received the standard diet, olive oil and vitamin B. Here absorption was by 'streams' of finely divided fat within the epithelial cell on either side of the nucleus. The supranuclear area was free of fat. In a few instances 'drops' were seen at the apices of villi, 'streams' being found in the sides. Fine fat droplets were also seen in the reticular tissue in the centre of the villi.

The difference between the two appearances was so striking that a glance with low power enabled the authors/

authors to tell with certainty whether an animal had been fed on the vitamin-rich or vitamin-free diet.

In a second series, cod liver oil (rich in Vitamin A) was substituted for the olive oil. Here when the diet was supplemented by Vitamin B absorption was entirely by 'streams.' With the omission of Vitamin B, drops were found in the cells at the apices, and a few streams were found at the sides of the villi.

Fat absorption, therefore, seemed to be most efficient in the presence of both, although, of the two vitamins, Vitamin B was the more important one to ensure efficient and rapid absorption of fat.

It is of importance to note that the effect was immediate. The experimental animals were normal and had not been subjected to vitamin deficiency prior to the experiment.

Almost all previous observers who had described fat within the epithelial cells during absorption, had seen only the fairly large drops. This was explained by Mottram, Cramer & Drew as being due to the fact that the fat used for feeding was usually olive/

olive oil, lard or bacon fats - fats free from the water-soluble Vitamin B and containing little if any of the fat-soluble Vitamin A.

Schäfer (1912) had stated that puppies and kittens fed on milk showed a different type of fat absorption. With Osmic acid darkly stained streaks were seen extending from the interepithelial spaces to the borders of the central lacteal. It seems significant that here the animals had been fed on milk which contained both vitamins.

Mottram, Cramer & Drew also noted that in rats receiving a vitamin-free meal, relatively large masses of fat were sometimes seen lying in the lumen of the intestine. This in itself seemed to suggest that the digestion of fat was less efficient and less rapid in the absence of vitamins.

Cramer & Ludford (1925) made use of these facts in investigating the role of the Golgi apparatus in fat absorption.

Irwin, Steenbock & Templin (1936), in experimental work, found that although a deficiency of Vitamins/

Vitamins A and B or D seemingly caused a decrease in rate of absorption of fat, this was probably due to general impairment of nutrition rather than any specific effect upon fat absorption.

This is rather in accord with Frazer's recent conclusions (1951) that, except for Choline, none of the known vitamins play any direct part in the mechanism of fat absorption.

Richter and his colleagues (1938) found that on a Vitamin B deficiency diet, rats showed a remarkable craving for fatty food.

Other effects of vitamins, therefore, appear to be more on the metabolism than on the absorption of fat.

Mahler & Nonnenbruch (1932) showed that, under the influence of stimulating substances such as paprika, etc., which cause an intestinal hyperaemia, fat absorption as seen in histological preparations, was more intense.

Species:

Some herbivorous animals are known to absorb fat much/

much more slowly and less efficiently than others.

Frazer and his colleagues (1945) found that guinea-pigs did not readily absorb fat from the intestine, and did not normally show an increase of fat particles in the blood during absorption. Such fat particles as occurred seemed to differ in structure from the usual chylomicron, as they did not flocculate with lecithinase.

Cook & Thomson (1951) in a comparative study of fat absorption, found that in rats and rabbits, on a diet containing 16.6% olive oil, absorbed respectively, 92 and 94%, which is absorption of the same order as that observed in man. Guinea-pigs at the same dietary level absorbed only 77%. Rabbits apparently absorbed cholesterol more readily and more efficiently than the others.

Stryker (1941) had similar results with liquid paraffin. It appeared that in the guinea-pig the oil was not absorbed so rapidly as in the rabbit and the rat.

Differences in the range of fat absorption and times taken for maximal absorption, etc., are discussed in the chapter relating to the sites of absorption.

Age:/

Age:

Sobel, Besman & Kramer (1949) have stated that newborn children absorb fats, and especially Vitamin A in oil, much less efficiently than children of one year of age or more.

Morales and his colleagues (1950) found that when premature infants were placed on low or high fat diets the percentage absorbed remained the same. (Reducing the particle size, however, greatly increased the amounts of fat absorbed).

Becker, Meyer & Necheles (1950), using the technique of chylomicron counts in finger blood, made a comparison between groups of young and old people. The latter maintained elevated chylomicron counts for many hours.

Chylomicron counts and nephelometric determinations on serum removed after ingestion of fatty meals indicated slower absorption of fat in aged than in young people (Marder, Becker, Maizel & Necheles,1952).

In the realm of animal physiology, it has been shown that age may affect the sites of fat absorption - the pH of the stomach contents of the young mammal being/

being able to potentiate the lipase present and thus enable breakdown, and perhaps absorption, to occur in the fundic or pyloric portions in some animals.

That it might affect the mechanism of absorption and the histological picture has been inferred in the writings of Schäfer; though Mottram, Cramer & Drew have indicated that this may be an expression more of dietary influence than one of age.

At the level of our present understanding, therefore, it seems true to say that diet, species and age differences may have a considerable influence on the sites, amount and even mechanism of fat absorption.

4. Other Sites of Fat Absorption:

The main absorption of fat occurs in the small intestine, and most of the histological work done on the subject has been confined to this region.

There is evidence, however, that in certain circumstances, some absorption may occur from other sites.

a) Stomach:

Although both Verzár (1936) and Frazer (1940) denied the likelihood of fat absorption from the stomach, on the grounds that normal gastric acidity precludes lipolysis, several papers have appeared to indicate that some absorption may occur from this site.

The most pertinent observations were those of Mendel & Baumann (1915), but older writers, too, made valuable suggestions in this field. More recently the experimental work of Platt and his colleagues (1954) throws an interesting sidelight on the subject.

The earliest histological studies were those of von Kölliker (1856), who noted that considerable numbers/

numbers of fat droplets were frequently seen in the epithelial cells of the stomach wall, in kittens, young dogs and mice. Although this was denied by Cunéo & Delamare (1900), Kischensky (1902), observed fat droplets in the gastric mucosa after feeding milk, olein, or oleic acid, to kittens aged fourteen hours to four months. His results were the same whether osmic acid, Flemming's solution, or scarlet red were used to stain the sections. In similar studies, Wuttig (1905) described these droplets in the fundic, as well as the pyloric region, after the ingestion of fat. They were fine in the cells of the epithelium and larger in cells more deeply situated. Lamb (1910), using Weigert's method for myelin staining, found fat globules were plentiful in the gastric mucosa of suckling kittens.

These findings were confirmed by Weiss (1912), who stated that the frequency of particles tinted by fat stains in the epithelium of kittens and puppies, increased from fundus to pylorus. Greene & Skaer(1911) agreed. These investigators failed to note that unless the meal given is large, the fundic epithelium may not come in direct contact with the ingesta, a fact which may account for the infrequency with which this/

this epithelium showed the particles.

Weiss believed that the mucosa lost its power of fat absorption early in life, the fundus experiencing this loss first.

On this point, however, Greene & Skaer (1911-12) brought considerable evidence to show that, although the process was much less active, fat was absorbed from the stomachs of old as well as young rats, cats and dogs.

In the descriptive part of their text, these authors recorded that the borders of the cells became filled with small droplets which gradually increased in size and number until the whole cell was filled with them. They made the significant observation that the number of droplets found in the cells bore a proportional relationship to the length of time the fat had been in the stomach. A maximum loading was noted six to fifteen hours after feeding. The epithelium of all regions of the stomach was involved except the stratified squamous epithelium lining the rat's upper stomach.

Hirayama (1922), not surprisingly, found that the/

the time taken for the appearance and gradual increase of the globules in the cells, as well as their decrease to the normal resting condition, varied with the species - the higher vertebrates taking two to twenty-four hours for completion, their maximum point being at 14-15 hours, while the lower vertebrates took a much longer period, such as 12-90 hours. He also found indications that the function, though present in old and young, decreased with age.

It seemed then that the histological appearance strongly resembled the picture obtained during intestinal absorption of fat.

Ogata (1881), Volhard (1901), Hull & Keeton(1917) had earlier pointed out that, while gastric lipase might not ordinarily be important, because of its sensitiveness to acid, it might bring about considerable hydrolysis when gastric acidity was low, and especially when fat was present in an emulsified form (as in milk, cream, yolk of egg, etc.).

Since both these conditions prevail in the young animal, it would not be altogether unexpected if, from the point of view of either the lipolytic or partition theory, absorption did, in fact, occur.

The/

The physiological data on the subject, however, failed to confirm this.

Klemperer & Scheurlen (1889) ligated the dog's intestine below the pylorus, and, after the introduction of a weighed quantity of fatty material, tied off the cardiac end of stomach. Excision and analysis, after three to six hours, recovered 99.5 per cent of the triolein, oleic acid or olive oil administered. These authors, on whose findings subsequent teaching was based, concluded that neither fats nor fatty acids were absorbed from the stomach.

The real problem seemed to hinge, not on whether fat appeared in the cells, but whether it was transported to lymphatics and to the systemic blood.

Von Kölliker (1856) doubted whether any fat left the stomach through its walls, since his own macroscopic examination of the regional lymphatics never showed them to be filled or opalescent.

Hirayama (1922), on the other hand, described fat globules present microscopically, not only in the epithelium and "hystiocyten" of the mucosa, but also in the submucosa, muscular layer, and free or within/

within "hystiocyten" in the lymphatic vessels of the stomach in kittens and frogs.

This again was opposed to the experimental findings of Mendel & Baumann (1915) who earlier attempted to elucidate the seeming contradictions. They confirmed many of the older observations, especially those relating to age, and found the distribution of globules in the epithelium to be far from uniform. In cats and dogs there was no alimentary lipaemia following the administration, under laparotomy, of 50 c.c. of peanut oil emulsion. Although they claimed to have proved that the operative measures did not in themselves influence the blood fat content, peristalsis was inhibited, and therefore, presumably, all forward transmission halted, of absorbents normally dependent on this movement within the wall. However, alimentary lipaemias were stated to occur following the introduction of fat emulsions to the intestine, under similar operative conditions.

In experiments with stained fat, introduced similarly to the ligated stomach, thoracic duct lymph failed to show any trace of the dye. It was also significantly absent from bile and blood.

They concluded that the histologically demonstrable/

strable fat passed into the epithelial cells of the stomach due to its preferential solubility in the cell lipoids, but that none passed through the submucosa into the blood or lymph streams.

Borrow & Platt's recent work (1950-1954) on the behaviour of food in the rat's stomach (especially of mother's milk in the stomach of the suckling rat), while not directly concerned with the absorption of fat, has interesting implications in this connection.

These workers noted that, except for a small soft portion in the pyloric funnel, the contents of the stomach of the breast fed infant rat can be shelled out of the viscus as a "bean-shaped, coherent mass." Sections showed that this was arranged in layers, as Grützner (1905) had previously described.

But, whereas Grützner found in the adult rat, that the last portion eaten was surrounded by shells of earlier portions, the stratification in the suckling rat's stomach was reversed. A similar effect was seen in the weaned animal fed on cow's milk; the portion of milk last taken surrounded the clot already formed.

These authors found that the first apparent change/

change in the milk in the stomach, was a separation into curds and whey, almost all of the latter from cow's milk, leaving the young rat's stomach quickly, whilst some of the 'cheese' from the same feed might still be in the stomach after four hours.

Preparations made of stomach contents of baby rats maintained at the breast, showed the remains of a clot from a feed taken more than twenty hours previously. Normally, the emptying of the stomach, apart from the departure of the whey fraction, depended on erosion of the pole of the clot in the antrum by regurgitation of duodenal juice.

Seckel & Kato (1938) had previously noted that the stomachs of rat pups were filled with milk clots at practically every hour of the day.

The stomach contents after a breast feed taken on an empty stomach showed no stratification, and more of a milk feed left an initially empty stomach than when added to material already present, since some fluid silk passed through without curdling.

It seems that only a small fraction of the fat separates in the whey, and would therefore be expected to leave the stomach quickly. (Professor Platt, in personal communication to Dr. H.S.D.Garven).

It is possible therefore that some of the fat remaining in the residue (especially if present in the finely divided form of milk fat) may be afforded facilities for passage through the wall by its long stay in the stomach, and by its enforced proximity and contact with the epithelium as it lies in the typical bean-shaped curd, moulded to the shape of the viscus.

Absorption of lower fatty acids is known to occur from the rumen of the ruminant stomach.

Volatile fatty acids are the chief nongaseous products of bacterial decomposition of cellulose in the sheep rumen. Absorption of these is not delayed till the small intestine is reached, but occurs in the stomach itself. (McAnally & Phillipson, 1943; Barcroft, McAnally & Phillipson, 1944; Elsden & Phillipson, 1948; Masson & Phillipson, 1951,1952; Phillipson, 1952-1953).

The veins draining the rumen, especially its lower wall, were shown by these workers to contain a higher concentration of these fatty acids than that in the general circulation.

Absorption of these fatty acids occurred but

to/

to a lesser extent, from the pig stomach.

Convincing proof of the possibility of direct absorption from the rumen of lambs was sought by Barcroft and others (1944), by the administration of radio-opaque material, capable, if absorbed, of being secreted by the kidney. Sodium ortho-iodo-hippurate inserted into a rumen from which all other paths of exit had been barred by surgical treatment was found, by X-ray examination, in considerable quantities in the bladder, and correspondingly, iodine was present in the urine.

b). Large Intestine:

Although it is generally agreed that maximum fat absorption occurs at the junction between the upper and middle thirds of the small intestine, fat can be demonstrated in the epithelial cells throughout the length of the jejunum and ileum.

Most writers assume that fat has been mostly absorbed by the time it reaches the caecum (Levites, 1906), and that no fat whatever seems to be absorbed there (Verzár, 1936).

However, here, as in the case of the stomach, there have been papers which approach the question from varying, and in some instances, intriguing angles, and throw doubt on the above assumption.

It is well known that the functions of the colon consist of storage and propulsion of the faecal matter, secretion of mucus to facilitate its passage, and reabsorption of water. There is, however, additional experimental evidence to indicate that, from caecum to anus, definite amounts of protein, carbohydrate, salts, certain metals, anaesthetics and drugs may be absorbed (Short & Bywaters, 1913; Curry & Bargen, 1935).

In/

In this connection it seems important to remember that embryologically, and functionally, the proximal and distal halves of the colon may be regarded as dual organs. Storage takes place in the distal portion of the transverse colon, in the descending colon, and in the sigmoid flexure, which develop from the hindgut. The caecum, ascending colon, and proximal part of the transverse colon, those parts which, together with the small intestine, develop from the midgut, are regarded as the absorptive part, and may, in some respects, share its functions.

Czerny & Latschenberger (1874) found a rather large absorption of fat emulsion from a blind pouch of the human large intestine.

In a similar patient, in whom almost the whole large intestine formed a closed tube, Kobert & Koch (1894) reported that emulsified fat was absorbed slowly in small amounts from this pouch, while non-emulsified fat was hardly absorbed at all. In the light of Frazer's later suggestion that fat is absorbed from the small intestine as a fine emulsion, this observation is interesting, especially at the lining epithelium of the proximal colon is seen to have a striated/

striated border.

Deucher (1897),and other workers, reported a small absorption, in patients kept on a fixed diet, and at intervals given enemata of fat. Their results have been criticised by subsequent workers on the grounds that fat was being given by mouth as well as by rectum.

Boyd & Robertson (1906) believed that considerable fat absorption resulted when a large amount of fat was used in the enema. This, however, may have been caused by regurgitation through the ileo-caecal valve, and consequent absorption from the small intestine. In addition, they were using the unsatisfactory 'wash-out' method, and felt justified in concluding that the fat which they failed to recover in the washings had been absorbed.

Langdon-Brown (1911) made an unusual contribution to the literature on the subject by reporting his observations on a case of filarial chyluria. Urine, which on ordinary diet was quite milky, and on fat-free diet merely opalescent, showed no increase in the ether-soluble bodies after the administration rectally of 120 c.c. of pancreatized olive oil. He concluded/

concluded that none, or little, of the fat had been absorbed by this route - a view with which Short & Bywaters (1913) completed agreed. The criticism could be made, however, that he had no guarantee of the fineness of dispersion of his emulsion, and also, since his case was pathological, that other lymphatics, for example those draining the large intestine, might have been blocked. Fat transport was obviously already greatly disturbed.

The most reliable experiments remain those of Munk & Rosenstein (1891), in which it was possible to estimate the fat in the chyle of the thoracic duct. The lymph collected from the lymphatic fistula showed a rise in fat content representing an absorption of 3.7 - 5.5% of the fat administered by enemata. Allowing for the fact that, even when fat is given by mouth, only about half of it is recoverable from the lymph, these figures may perhaps be doubled to represent actual absorption.

Another valuable line of approach is that of chylomicron studies, and this method was adopted by Nakashima in 1914.

Observations on the histology had been recorded previously/

previously by Eimer (1869), who noted that "in the small intestine and in the upper part of the large intestine, the epithelial cells of the mucosa were filled with fat droplets, first in the outer, superficial zone, and then in the basal parts of the cells."

Wuttig (1905) was also convinced, from histological evidence, that absorption could take place in the colon.

Nakashima, however, combining histological studies of the intestinal epithelium, with chylomicron counts in the blood of the mouse, found no evidence of such absorption, where the preliminary precaution of separating small from large intestine by ligation of colon was carried out before rectal administration of the milk. His compatriots, Yamakawa & Fujinaga (1929), in somewhat different experiments in dogs, found evidence that emulsified fat and lecithin introduced into the ligated large intestine, was absorbed to a great extent over the course of twenty-four hours. Further studies satisfied these authors that the major part of the fat left the large intestine in the lymphatics and was traced in the thoracic duct. Only a small portion went directly into the blood stream.

Both/

Both sets of experiments, by ligating the colon, eliminated the possibility of regurgitation through the ileo-caecal valve, and consequent absorption from the small bowel. The difference in results may have been due to the relative amounts, or the form, in which the fat was given, or to a difference in absorptive capacity of the bowel, inherent to the species concerned.

Little attention has been paid, in respect of this problem, to the fundamental differences which exist in the macroscopic and microscopic features of the various parts of the gut, in herbivorous, carnivorous and omnivorous animals. The proportional extent of these parts, and the related functional requirements, obviously vary with the dietary habits of the species.

Indirect evidence on the subject is provided by Sperry & Angevine (1932), whose work on endogenous fat excretion is discussed, in this connection, in a later chapter. Their finding that much larger amounts of lipids were excreted from fistulas of the ileum, in dogs, than were excreted in the faeces on a fat-free diet, seemed to force the conclusion that there was considerable absorption of lipids from the/

the colon. Experiments with caecostomies further indicated that reabsorption did not take place in the lower part of the small intestine itself.

More recently, however, Volk & Popper (1950), with the aid of the fluorescence microscope and frozen sections, were unable to demonstrate fluorescence, due to fat, in either the large bowel or its blood vessels.

Finally, as a postscript on the subject, it must be noted that clinical studies on patients, in whom almost all of the small intestine has been resected, have repeatedly shown that assumption, by the colon, of the capacity to absorb fat is among the adaptive changes which may take place.(Althausen, Doig and others (1950)).

The work of Barcroft, McAnally & Phillipson (1943,1944) already quoted in connection with absorption of volatile fatty acids from the sheep rumen, indicated that fermentation also occurred in the caecum, and that significant quantities of the resulting fatty acids were present in the blood draining that organ.

Similar/

Similar absorption appeared to take place from the large intestine of the horse, pig, rabbit, rat and dog (1947). In the rabbit these fatty acids were absorbed about three times as rapidly from the caecum as from the small intestine.

It seems established then that the bulk of fat absorption occurs in the upper jejunum, but the process may involve a much more extensive part of the tract, including, in some species, the wall of the stomach and the large intestine.

While the histological picture described by Frazer as due to the presence of neutral fat is seen in cells lining the small bowel, reports on the nature and route of absorption of sudanophilic inclusions in the gastric and colonic epithelium are equivocal.

In the light of Sperry's findings on endogenous fat excretion and reabsorption in the large bowel, and Phillipson's on the absorption of volatile fatty acids in stomach and caecum, it is suggested that some correlation between these biochemically determined facts and the Sudan-stained, histological picture is to be expected.

5. The Nature and Role of the Migratory Cells.

Few subjects can have baffled histologists for so long. Many papers have appeared on the subject, and contradictory views been put forward about both their nature and their role.

Slowly and painstakingly our knowledge of the nature of these cells has grown, but our understanding of their function, in spite of many and varied theories advanced, is still obscure and incomplete.

The small round elements among the epithelial cells were first noticed by Rindfleisch in 1861, and their migratory nature suspected by Eberth a few years later (1864). Many of the older writers saw them within the stroma of the villi, and described them as lymph-corpuscles (Fries, 1867; Watney,1876).

Arnstein (1867) was the first to record the presence of fat in these 'leucocytes' of the intestinal epithelium, and in fresh preparations, to observe their migration from the epithelium to the lumen of the intestine.

This finding was lost sight of, for a long time, and it became popular to regard them as migrating in the/

the opposite direction.

Zawarykin (1883) suggested that leucocytes took up fat droplets by phagocytosis from the intestinal lumen, and transported them between the epithelial cells into the lacteals.

Schäfer (1885), one of the chief investigators of the subject, agreed with Zawarykin in this, and pointed out that he had long regarded the "amoeboid cells which occur in large numbers in the mucous membrane of the intestinal tract" as the active agents in promoting the absorption of fat.

It is obvious from his further descriptions that he equated them in morphology to the lymphocytes found in the lymph nodules and Peyer's patches. He described their occurrence within the lacteals of the villi (especially near the tips), and was convinced that they passed into these vessels from the surrounding stroma. To account for their scarcity in the deeper lymphatics of the intestinal wall and the mesentery, he thought that, after reaching the interior of the lacteal, they disintegrated and dissolved in its lumen, thus setting free their contained fat.

Later/

Later (1912) Schäfer modified his view to the extent of admitting that the fat transfer took place from the epithelial cells, and not from the lumen of the intestine, by means of these corpuscles.

He still referred to lymph-corpuscles and leucocytes synonymously, and it is obvious that neither he nor Zawarykin made an accurate differentiation of the white cells involved. Their drawings did nothing to clarify this.

Wiemer (1884), Grünhagen (1887), and Heidenhain (1888) criticised and did not accept their views. This last writer, while admitting that leucocytes could take up fat from the intestine, ascribed to them only a minor role in its transport. He did, however, attempt to make some differentiation of 'leucocyte' types in the wall of the intestine. He showed that while some contained true fat droplets, others contained inclusions which blackened with osmium tetroxide and were insoluble in ether and stained with acid fuchsin.

With regard to the origin of these cells, Schäfer believed they arose chiefly from division of pre-existing lymphoid cells in the mucous membrane. He thought it/

it unlikely that they migrated from blood vessels, since he had rarely seen them within blood vessels, in the tissue immediately surrounding them, or in the act of passing out through their walls.

He equated them in function with those found in the trachea, bronchi and alveoli of the lungs, which ingest inspired carbon particles and transport them to regional lymphatics and lymph nodes. He thus regarded the processes of internal absorption, such as the activity of white blood cells in ingesting foreign substances, or dead tissues, etc., as being essentially similar to the process of fat absorption.

To substantiate these views, he cited many observations which tended to show the importance of amoeboid cells in the promotion of various absorptive processes. Chief of these were the observations of Metschnikoff (1883), who described the absorption of the tail of the batrachian larva by phagocytic leucocytes.

In many cases amongst the lower metazoa, Metschnikoff and others, showed that both ectodermal cells and also migratory mesodermal cells were directly concerned/

concerned with the inception of food particles which they believed might be submitted to a process of intracellular digestion.

Such transformation or breakdown of foodstuffs ingested by these migratory cells was supported by Oppel (1899), while the original idea that they served merely to carry the materials to the lymphatics, and perhaps to subject them to mechanical breakdown into fine particles, was defended by Moller in the same year. Munk (1884) was of the opinion that a synthesis of fats took place in the lymph corpuscles of the villi.

One of the most potent arguments in favour of phagocytosis as a factor in the transport of fat was provided by Bondi & Neumann (1910), and Bloor (1922), who found that fine emulsions of coloured or otherwise marked fat, when injected to the circulation, collected in those parts of the body where cells of the reticule-endothelial system were known to occur (e.g. liver, bone-marrow, spleen).

In this respect fat appeared to behave like particles of other foreign matter such as vital dyes.

It had become obvious, however, to several writers, that certain anomalies were not explained by any of these theories.

Apart from the finding that leucocytes were often relatively rare in the mucosa during digestion, and increased during fasting (Heidenhain, 1884), migratory cells were not found exclusively in the absorptive part of the digestive tract. Prenant (1896) saw "masses" of leucocytes invading the oesophageal epithelium in young slow-worms. Arnstein, as far back as 1867, had noted them in the oesaphagus and stomach of the frog, while Stohr (1884) made the same observation in the stomach epithelium of man. Much later, Onazaki (1936) described "phagocytic" cells in the subepithelial tissues in the colon of the dog.

Béguin (1903) was frankly sceptical about their supposed digestive or absorptive function, and made the suggestion that they were either taking nutriment to the epithelial elements, especially during fasting, or carrying useless substances across the intestinal wall in excretion.

Schäfer (1912), however, remained unshaken in his/

his theory, and the first decades of the twentieth century saw the publication of several papers which appeared to give him support.

Bradley (1912) provided some evidence that well-emulsified paraffin oils were apparently absorbed partly by leucocytes. Clark & Clark (1917) presented experiments in which migrating leucocytes were seen to migrate through the walls of nearby blood vessels to ingest and transport to lymphatics, fat particles injected to the tails of tadpoles. (Unfortunately, they did not at this time distinguish between the various types of leucocytes, and later work (1930) showed that lymphocytes and granulocytes were apparently inert, and only the macrophages and monocytes were engaged in this particular process.)

Ziegler (1921) showed that fine emulsions might be absorbed from the peritoneal cavity, probably by the phagocytic activity of leucocytes, and this was taken as added support for Schäfer's point of view.

Bunting & Huston (1921) found the blood lymphocyte count to be constant, though more lymphocytes entered the blood stream from the thoracic duct in twenty-/

twenty-four hours than were present in blood at any one time. They regarded the excess numbers present in the mucosa of the gastro-intestinal tract as sufficiently great to account for this otherwise seeming discrepancy.

A survey of the literature of the period suggests that the lymphocyte is a pluripotential cell capable of a varied differentiation depending on the specific stimuli of its environment. Thus Maximow (1916) and Danchakoff & Seidlein (1922), regarded it as capable of becoming a macrophage by stimulation to phagocytosis, just as Schäfer regarded it as assisting in the digestion of fat. Bunting & Huston (1921) thought it functioned as an agent in neutralising certain bacterial toxins in the alimentary tract. In this they were supported by Jordan & Speidel (1924), who also correlated the function of lymphocytes with general immunity reactions against toxic properties of bacteria in the intestine, and regarded them as capable of differentiating into eosinophils within the epithelium.

The latter writers further introduced the idea that eosinophils, probably arising thus from lymphocytes, passed into, and ultimately through the epithelium,/

ium, to disintegrate among the faecal contents of the lumen. Lymphocytes, as such, they believed seldom passed through the epithelium. Mottram, Cramer, & Drew (1922) studied the effect of greatly reducing the number of circulating lymphocytes by irradiating rats. When the animals received a large dose of screened radiation, no fat absorption occurred at all, even in the presence of the necessary vitamins. When they were exposed to smaller amounts of radiation, only absorption by 'drops' occurred, and none by fine 'streaks', whether or not vitamins were present. (See Chapter on 'Effect of Diet on Fat Absorption'.) This effect of radiation was interpreted as striking confirmation of Schäfer's views on the importance of lymphocytes in fat absorption, and, indeed, these authors were inclined to attribute to these cells, functions even more important than the transport of fat which Schäfer assigned to them.

For a time then, attention was focussed, almost exclusively, on the lymphocyte cell, but gradually an element of doubt began to creep into the literature.

Although the differential staining of white blood cells had been described by Ehrlich in 1879, and/

and a study of leucocyte types made by Hardy & Wesbrook (1895), it was some time before the discoveries were widely applied.

In addition, differences were found to occur with the species. Bunting (1922) showed that, in man, there were polymorphonuclear leucocytes with a neutrophilic staining reaction. In the rabbit the morphological and functioning equivalent had an eosinophilic (pseudo-eosinophilic) reaction, while in the guinea-pig this cell was amphophilic. In the rat and mouse the granules were undemonstrable by use of the ordinary stains.

This was obviously of importance in describing the free cells in the intestinal wall, and might account for many of the discrepancies that had previously arisen in the description and interpretation of results. It became increasingly obvious that many of the cells described had not been lymphocytes at all, and that in fact lymphocytes had little or nothing to do with the process of fat absorption.

Winiwarter (1930) found that prolonged feeding on an exclusively fatty diet had no effect on the number/

number of lymphocytes in the intestinal epithelium of mice. Indeed, he thought that only protein feeding resulted in an accumulation of free cells in the mucosa of the intestine, and that these were macrophages and eosinophiles.

The problem passed into a phase where there was little accord as to the nature and origin of the cells, and the direction of their migration. With regard to their function, it had been variously suggested that they served to carry fat, that they subjected it to any intracellular breakdown or synthesis (mechanical or chemical), and that they served either as a protective or an excretory mechanism. In actual fat, it was by no means certain that they did ingest fat. The chief criterion on which their ability to do this was assessed, was their positive reaction with osmic acid. As Leach (1938) later pointed out, many of the granules which appeared brown or yellowish-brown with this method did not stain with Scharlach Red and were, therefore, probably not true lipides. Many of the cells described by Zawarykin & Schäfer were undoubtedly in this category.

Goldman (1933), using his own modification of the Sudan staining technique, on material from mice, rats, rabbits and guinea-pigs, was unable to find fat in/

in lymphocytes, although he identified it in all other types of leucocytes. In the protoplasm of the polymorphonuclear cells he claimed to find two types of Sudan-stained granules which he called respectively, "integral" and "infiltrative." He claimed that the two types were bound up with each other, but that it was possible to destroy one without affecting the other. In the gut of the dog, he found 'lipoid' present, not only in eosinophils, but also in other members of the white cell series. He made the very important, but long over-looked point, that, in leucocytes, 'lipoid' material was normally present, quite apart from any question of their participation in the processes of fat absorption.

These matters required further elucidation, and Weill (1920) & Leach (1938), reinvestigated the free cells in the mucous and submucous coats of the intestine.

Using osmium tetroxide and chromic acid, Leach found it possible to distinguish three types of leucocytes in the intestinal epithelium of the frog. Lymphocytes were present between the epithelial cells and in the subepithelial connective tissue. The number/

number found during the various stages of digestion did not differ from that found during starvation. No fat was identified in them with Osmic acid. He thought it unlikely that they were involved in fat absorption or transport.

Eosinophils, although occurring in the epithelium, especially near the basal border, were more numerous in the subepithelial tissues. They decreased in number during digestion, and he was able to recognise them, in sections stained with Osmic and Chromic acid, by their highly refringent granules. With osmic acid alone these granules were often dark brown or even black, and it was obvious that many of Schäfer's cells were in this category.

Large phagocytic cells, with flattened, or crescentically-shaped nuclei, were occasionally seen to migrate through the striated border, and were present in the lumen of the gut itself. During digestion, he found a decrease in the number of these phagocytic cells. Vital staining with trypan blue indicated that they were members of the reticulo-endothelial system. Within them he found black droplets of varying size. These were quite distinct

from/

from the other brown-staining, cell inclusions such as red blood cell fragments, etc.. The number and size of the droplets increased during fat absorption although the number of the cells themselves diminished. Similar black droplets with osmic and chromic acids were present even after the animal had fasted for twenty-eight days. Leach was able, only occasionally, to see members of this series containing black droplets in the subepithelial tissue, or in the walls of the lacteals. When present they were invariably much smaller than those seen between epithelial cells.

It was significant that, after treatment with ether for 24 hours, the black droplets of these phagocytic cells disappeared, whereas the black-brown granules of the so-called eosinophils remained unaffected. Similarly, with Scharlach red, the oxyphil granules of the eosinophils were a light yellow colour, while the epithelial and phagocytic cells were full of red-staining droplets.

The results of his experiments seemed to indicate that the phagocytic cells migrated through the epithelium, that they took up fat just as they took up other particulate matter (e.g. vital dyes), due to their inherent phagocytic property and not because/

because they had any essential function to perform in fat absorption. This fat was discharged when they disintegrated within the lumen of the gut. Such phagocytosis and migration would, in Leach's opinion, involve a very minor inefficiency in fat absorption. The fat occasionally seen in subepithelial histiocytes was also noted by various other workers (Zawarykin, 1883; Weiner, 1932). These cells were definitely not lymphoid in character. Onazaki (1936) described them in the colon of the dog.

Leach concluded that there was no evidence that any type of leucocyte played an important part in the transference of fat from the epithelium to the lacteal - an opinion with which Drinker & Yoffey (1941) were in agreement.

Verzár (1936), while not excluding the possibility that they might serve some other, as yet not understood, physiological function, supported the view that the importance and rôle of the leucocytes was as a protection against the masses of bacteria in the lower parts of the intestine, and possibly also against toxic substances absorbed.

Wotten/

Wotten & Zwemer (1939) found occasional granular cells in the villi of the cat during active absorption of fat, but denied that their small number could account for much of the fat transferred.

These authors stated that most of them were eosinophils, which, even during the peak of fat absorption, showed no tendency to pick up, or carry, 'lipoid' material. They concluded that the possibility of fat transportation by histiocytes and other cells (as proposed by Schäfer) was slight. It must be added, however, that, since they described only methods using frozen sections stained with Sudan and haematoxylin, it is difficult to see how they determined, with any degree of certainty, that the cells involved were eosinophils, and not other types of polymorphonuclear leucocyte.

It will be seen that, while their function was still in doubt, the emphasis on the predominating cell type and the direction of migration had changed.

More recently, Volk & Popper (1950), in the work mentioned earlier, using the fluorescence microscope, found large fat droplets in the cytoplasm of monocytic cells in the lamina propria of villi one hour/

hour after the administration of corn oil..(It is of additional interest that vitamin A fluorescence was found to predominate after more than 24 hours, in the 'round cellular elements in the lamina propria, and some in the lymph vessels of all layers of the intestinal wall'.)

Other methods for the chemical identification of the granular substance in polymorphonuclear leucocytes seemed indicated.

Baesich (1935) found lipoid granules to be a constant physiological constituent of the human blood granulocytes, and the appearance and arrangement of these granules, as stained with Sudan III in the various leucocytes were identical (except for colour), with those of the granules stained by the customary blood staining methods or by the oxydase reaction.

Sheehan (1939), McManus (1945), Wislocki & Dempsey (1946), and Eranko (1950) were among those to employ the Sudan Black technique for the staining of these granules in blood leucocytes. Pearse (1953) points out that although this stain is far from specific for phosphatides, these are the most common lipids/

lipids demonstrated by its use, after the exclusion of neutral fat.

Berenbaum (1954), by staining tissues in 2 per cent Sudan Black in acetone at 37 degrees C., obtained results suggesting that his method stained lipids bound to protein, and possibly to carbohydrate and nucleic acids. Eosinophil granules were among the structures stained by this and other related techniques.

Although we have come a long way from the original conception of these cells we are still far from understanding their function. It would appear that, instead of taking up fat during absorption, they are actually travelling in the reverse direction, and more than one cell type may be involved.

In this connection it is of note that Loeper & Marchal (1922) found, after the ingestion of bouillon, as many as 1880 leucocytes (80-90% polymorphonuclear leucocytes) per cubic millimeter, present in the gastric contents.

Bunting & Huston (1921) believed that large numbers of "lymphocytes" were constantly passing through the intestinal mucosa and entering the lumen of/

of the intestine.

Ohno (1930) advanced the theory that "lymphocytes" in the lumen of the intestine were powerful activators of the digestive enzymes, and thus assisted in the digestion of protein, starch and fat by the pancreatic juice.

It has long been known that fluctuations in the blood leucocyte count may occur throughout the day, and for a time it was supposed that these might be due to digestive activity.

Later workers, however, found little evidence of a digestion leucocytosis. (Sabin, Cunningham, Doan & Kindwall, 1925; Garrey & Butler, 1932; Kennon, Shipp & Hetherington, 1937; Casey, 1940).

Sabin and her colleagues found that the white blood cell count was raised in the afternoon independent of food intake, and that the increase was due to changes in the numbers of neutrophils present. It is generally agreed that these increases are due to a redistribution of white blood cells in the vascular system rather than to the formation of new leucocytes (Sturgis & Bethell, 1943).

Such/

Such a conclusion raises interesting speculations as to whether or not a simultaneous fall or rise may occur in the numbers of granulocytes present in the intestinal wall.

In summary, the most recent assessments describe these cells as predominantly eosinophils, while some are regarded as phagocytes of reticulo-endothelial origin.

Work on blood leucocytes indicates that the integral granules are sudanophilic, and this suggests perhaps why migratory cells in the gut wall were mistakenly regarded by earlier writers as playing a purposive role in the transport of absorbed fat.

While the direction of migration seems established, the exact role of this and the question of its periodicity in relation to the digestion phase, are matters still open to doubt.

In view of the possibility that the cells in the gut wall are chiefly polymorphonuclear granulocytes, and that their integral granules are lipid in nature, one further possibility suggests itself to the mind - that these cells may be responsible, by their disintegration within the lumen of the gut,/

gut, for a considerable fraction of the faecal fat.

It therefore seemed relevant to review present knowledge on endogenous fat excretion.

---

6. The Endogenous Fat Excretion:

It has long been recognised that the faeces contains a fatty substance (Home : 1813).

For some time it was generally assumed that this represented an unabsorbed residue of the fat from food.

Thiry (1864) was the first to cast doubt on this, with his, now classical, Thiry Fistula. Many subsequent workers made use of his and Vella's (1886) techniques. Among these were Gumilewski (1886) & Rohmann (1887), who succeeded in obtaining continuous, small secretions from low fistulas during fasting. An isolated high loop filled up in sixteen days with sixty grams of faecal material (Hermann, 1890). Examination of this by Ehrenthal (1891) demonstrated the presence of fats, soaps and cholesterol. His own observations confirmed that the secretion was continuous, and that globules of fat were present microscopically. The contents of the intestinal ring were found to have the same composition as hunger faeces (Voit, 1892). In addition to ash and nitrogen, there was always 30-36 per cent fatty material, of which up to a third was neutral fat,/

fat, a half to four fifths, free, fatty acids, and a tenth to a third, soaps. He attributed this material to intestinal secretion, and, on the basis of the concomitant nitrogen content, which was too low, and ash, which was too high, concluded that it was not of cellular origin.

Müller had earlier observed somewhat similar percentages of fatty material in the faeces and meconium of fasting dogs (1884). The total lipid excretion of professional fasters was found to differ little during food and fasting periods (1893).

Attempts to determine its origin led to the examination of the intestinal epithelium and mucosa (Schmidt, 1897; Ewald, 1883; Moore, 1904; Noll,1910). These estimations revealed a lipid content varying from 4 to 6 % (based on the dry weight of the mucosa).

Various workers advanced the theory that the faecal fat might represent a leakage from the plasma, and might subserve some lubricant or metabolic function (Niemann, 1912; Hutchison, 1919-20).

This was the position when Bloor and his colleagues began their long series of investigations into the/

the exact nature, source and function of the endogenous fat. The faeces were known to contain fatty material, consisting mainly of fatty acids, and their salts (soaps), a smaller amount of cholesterol and its derivatives, and a little neutral fat. This material, in composition and amount, was little affected by the fat of the food (Holmes & Kerr, 1923-4; Angevine, 1929). Its similarity to that of the plasma and fat depots had been demonstrated (Sperry & Bloor, 1924; Eckstein, 1925).

Many theories were advanced as to its origin and function, and amongst these were the four possibilities systematically investigated by Sperry and his co-workers (1924).

They confirmed its constancy independent of the diet, and demonstrated conclusively that bile was not its source, since the average lipid excretion was more than doubled when bile was excluded from the intestine (1926-7). About 40 per cent of the lipids excreted by dogs on a lipid-free diet were contained in the bodies of bacteria (Sperry, 1929), and only a negligible portion was present in soluble or suspended form. A comparison of the lipid content of the mucosa and the amounts of lipid excreted/

excreted by such dogs seemed to indicate that desquamated intestinal epithelium was probably not an important source. About 12-14 per cent of the entire mucosa would require to be desquamated per day to account for the average lipid excretion in the normal dog (~~1932;~~ Gardner & Gainsborough, 1930; Sperry, 1932). There remained the suggestion that it might represent a secretion across the intestinal wall.

Their subsequent investigations showed that the excretion originated principally in the small intestine. Relatively large amounts of lipids were secreted here, and a considerable portion of them were apparently reabsorbed in the colon of the intact animal. The remainder, together with the relatively small amount secreted into the large intestine, probably made up the endogenous lipid excretion.

The finding of considerable amounts of sterols seemed to indicate that the lipds present in the large numbers of bacteria had to a considerable extent at least been adsorbed on, rather than synthesised by bacteria (since all the available evidence pointed to the probability that bacteria were themselves sterol-free).

Regarding/

Regarding the physiological significance of this endogenous lipid excretion, Sperry suggested that it either represented leakage from the plasma for lubrication of the intestine, or acted as solvent in the removal of undesirable or excess sterols from the organism.

Apart from this idea of "leakage," no agent has been suggested in the transfer of the lipid material from the plasma across the intestinal wall, although Schönheimer & von Behring (1930) did draw attention to the large number of lymphocytes in the intestinal lumen, which were rapidly destroyed by the intestinal ferments. They suggested, in a private communication to Sperry & Angevine (1932), that the lipids found in the intestinal excretion might, to a large extent, be contained in these cells. Sperry & Angevine (1932) examined a number of smears of the excretion, and saw occasional, strongly-stained particles with scarlet red, but were unable to state whether these were free droplets of fat or were contained within cells. None of the bacteria seen in these smears stained positively with the dye.

Some of these findings were confirmed by other workers/

workers on the human subject.

Krakower (1934), in studies on twelve normal subjects administered carmine with the food at the beginning and end of the experiments, and was able to ascertain, from the iodine absorption numbers, that faecal lipids did not have the characteristics of the dietary fat, and were not greatly influenced by it in type and amount. He concluded with Bloor and his co-workers, that lipids from the blood plasma were probably excreted through the intestinal wall. Doubilet & Reiner (1937) found that the ileum in the human subject secreted a fluid containing 2% of lipids. This seemed to confirm that the lipid secretion, whatever its function, originated principally in that part of the bowel where absorption was still taking place.

In 1942, Nunez & Bargen, studied the excretion of fat by the intestines of fourteen patients, two of whom were normal, some had ileocolostomies with or without resection of the right part of the colon, and some suffered from thrombo-ulcerative colitis, involving part or all of the colon. All of them received a fat-free diet, with carmine and charcoal as indicators of the beginning and end of the experiments./

ments. The number of observations was small, but their results suggested that excretion of fat by the intestine was greater in the normal subject than in those subjected to resection of the right part of the colon, or with extensive ulcerative colitis.

Finally, Bernhard, Ritzel & Hug (1952) used fat in which all the components were labelled with deuterium. The lipids in the faeces consisted of a mixture of ingested fat and body fat which had been excreted into the intestinal lumen.

In summary, the endogenous fat excretion appears to originate chiefly in the small intestine. A considerable portion of it is apparently reabsorbed in the colon. The remainder, together with the small amount secreted in the large intestine, and any residual ingested fat, make up the lipids of the faeces.

While many possibilities regarding the agency in the transfer of the endogenous fat excretion across the intestinal wall have been excluded, the exact nature of this and the function subserved are still largely undetermined.

7. The Role of the Regional Lymph Nodes:

Free cells containing fat droplets are found in the mesenteric regional lymph nodes following the ingestion of fat. These have been variously described as "small round cells, with large nuclei" (Kischensky, 1902), and in general terms, as macrophages or phagocytic cells (Bloor, 1922).

In addition, free, extracellular droplets of fat are seen in the loose lymphatic tissue, and if a reasonable quantity of fat has been ingested, these may assume flood-like proportions. Jéker (1936) found that the mesenteric lymph nodes were enlarged six hours after ingestion of fat. Sudanophilic material in the Sinuses was negative with Fischer[1] stain, and this was taken to indicate that only neutral fat was present.

In common with many of the other findings during fat absorption, there has been considerable difference of opinion as to the relationship of the mesenteric lymph nodes to fat transport and metabolism.

The fluid coming from the different drainage areas for filtration in regional lymph nodes is far from/

from uniform, and, it was suggested by the older writers that, just as the lymph nodes are placed in the pathway from lung or arm to carry out specific functions varying with the region concerned, so the nodes of the mesentery are placed in the pathway of the lymph from the intestine to play some active, physiological part in the phenomena of nutrition.

Munk (1884) thought that the synthesis of neutral fat from its split products occurred to some extent in the lymphatic glands. Loevenhart (1902) demonstrated that both lymph and lymphatic glands possessed marked lipolytic activity (though not great when compared with that of the liver and pancreas).

Poulain (1902), acting on the supposition that some specific function was involved, carried out experiments on young dogs at different stages of digestion. He found that the small quantity of fat in the sinuses at the beginning of absorption had greatly increased after four to six hours, and was partly contained in migratory cells.

Noting, nevertheless, the irregular distribution of fatty material coloured by osmic acid in the inter-nodal tracts, and the evident disproportion between/

between this, sometimes small, amount and the contrasting large quantity of apparently milky lymph which, even to the naked eye, filled the node during intestinal digestion, he devised experiments to elucidate this anomaly. Eventually he interpreted it as due to the presence of saponification derivatives of fat which were dissolved out of the lymph in the preparation of the sections.

His analyses led him to conclude that a series of decompositions and resyntheses of fat occurred in the mesenteric nodes, under the influence of a lipase of nodal origin - these "multiple transformations" having as their final goal the "chemical identification of the ingested fat with the special fat of the species".

In the hours following the active phase of intestinal absorption, the amount of free fat in the sinuses gradually diminished and disappeared. Similarly, the macrophages with fatty granules in their cytoplasm became fewer, and eventually, during physiological fast, rare. When the fast was prolonged the lymph remained free of fat, but great numbers of macrophages made their reappearance. These cells appeared to have mobilised the reserve fat from the mesentery -

a/

a similar resorption of fat having occurred throughout the economy. It was Poulain's opinion that, when alimentation became insufficient, reserve fat, brought thus to the lymph nodes, was again emulsified and decomposed by the lipase and pushed out into the lymph stream in minute droplets and thus made readily available for combustion. In this way he assigned a regulatory role in fat utilisation and storage to the regional lymph nodes.

Stheeman (1910) believed, as Poulain did, that there was a reciprocal relationship between the mesenteric and other lymph nodes - the peripheral nodes, in starvation, becoming loaded with fat mobilised from the fat depots. He studied the lymph nodes of dogs, and concluded that during digestion the fat was emulsified and changed chemically in the mesenteric lymph nodes.

Holthusen (1910) thought that all lymph nodes in the body, not only mesenteric, became filled with fat during absorption. Later work failed to confirm this.

Fiessenger & Marie (1909) reported a fat-splitting ferment in lymphocytes, and a proteolytic ferment/

ferment in the polymorphonuclear leucocytes. This study, though not of immediate bearing on the problem, was regarded as significant.

Czerney (1907) found a marked enlargement of lymphoid tissue in over-sized children.

In those cases where 'over-nutrition' produced adiposity there was an enlargement of lymph nodes. Regulation of the diet in adiposity was accompanied by a diminution in size of the lymphoid organs.

This suggested another line of approach to the question of lymph nodes in their relation to fat metabolism.

Recognising that in early life the amount of lymphoid tissue was relatively great, and diminished with age, and believing that the most important differences to which the young animal was subjected were those connected with diet, Settles (1920) carried out an investigation on kittens, in which he found that the animal fed for some months on a high calorie, high fat diet showed an increase in the lymphoid tissue especially of the gastro-intestinal tract, greatly in excess of that found in the kitten kept on a normal calorie , normal fat diet.

It is well known that an increase in the specific function of a tissue frequently results in an increase in its size. Lefholtz (1923-4), following up Settles' experiments, worked on the assumption that if lymphoid cells were concerned in absorption and transportation, and perhaps digestion of fat (or sugar or protein), it was reasonable to expect that the lymphoid organs would show an increase in size with an increase in the amount of food ingested. He carried out experiments to discover whether Settles' increase was due to the excess calorie value of the diet given, or wholly to the specific action of the fat. The effect of long-continued feeding of diets rich in sugar and protein on the size of the lymphoid organs of kittens was studied and compared with results obtained by feeding a diet rich in fat.

The mesenteric lymph nodes showed a consistent increase in size with a diet rich in fat, and were relatively unaffected by a normal calorie diet rich in sugar or protein. A high calorie diet in which the excess calories were provided in the form of sugar or protein did give a marked increase in the size of the lymphoid tissue of the gastro-intestinal tract, but the uniformly larger size of the mesenteric lymph/

lymph nodes in the animals receiving the high fat diets suggested a possible relation between the size of these organs and the amount of fat brought to them by the lymphatics.

Another interesting fact which emerged from this work was that the total weights of lymphoid tissue of the body were fairly uniform for the animals on the different diets. This seemed to suggest the possibility of a reciprocal relationship between the lymphoid organs, so that if one was unusually large, the others would be smaller than average.

Lefholtz concluded that the amount of lymphoid tissue in the bucco-pharyngeal cavity and in the intestine, was regulated both by the calorie content, and also specifically by the fat content, of the diet.

An extensive literature has grown up round the changes in the lymphoid tissue during malnutrition and starvation, and it would appear that, while this tissue is more sensitive than the rest of the body to shortage of food in general, it is especially sensitive to a dietary deficiency of fat (Jackson, 1913, 1915). The lymph nodes respond by a characteristic atrophy of the lymphoid elements, in which there

is/

is a decrease in the number of lymphocytes. Adequate refeeding after ~~unlimitation~~ inanition usually leads to prompt recuperation on the part of the lymph nodes, with increase in weight, active mitosis and rapid recovery of the normal structure of the lymphoid tissue.

All of these researches lend weight to the idea that lymph nodes have some active part to play in the processes of fat metabolism.

Dabelow (1930-31), in a comparative histological study of the changes in the mesenteric lymph nodes of the rat, mouse, guinea-pig and cat during fat absorption, found a definite cycle of changes during digestion. In the passage of chyle, the cells of the reticulum, especially those of the loose lymphatic tissue of the sinuses, became loaded with fat, and many of these became detached as free macrophages. When the passage of chyle was complete, the fat content of the nodes diminished, though it never completely disappeared. The lymphocyte did not appear to take part in the storage of fat, but it was noticed that, after a short fast, there was diminished multiplication of cells in the germinal centres. With prolonged fasting, the sinuses and medullary cords again/

again became as rich in fats as during a period of intensive feeding, but the germinal centres tended to disappear completely. Sudden feeding with milk or cream following such a fast tended to wash the sinuses clear of their remaining free cells. After physiological fasting, multiplication of macrophages and of lymphoid cells resumed half to one hour after a meal.

Drinker & Yoffey (1941) confirmed Dabelow's other finding that during digestion the only lymph which contained fat in any amount was that passing through the mesenteric nodes. In two dogs fed with cream and eggs, thoracic duct lymph contained from ten to twenty times as much fat as the cervical lymph from the same animals.

Weiner (1932), with Munk, thought that in fat absorption in mammals, only fatty acids were absorbed through the epithelial cells, and these were synthesised to neutral fat extracellularly, in their passage through the sinuses of the mesenteric lymph nodes. He thought that the lymphoid structures of the intestinal mucosa were also probably capable of some synthesis of neutral fat.

The appearance of increased quantities of fat in lymph during starvation was confirmed by Rony, Mortimer & Ivy (1932). These investigators noted the presence/

presence of lipids, sometimes in considerable amounts, in the thoracic duct lymph of dogs subjected to fasts of from 2 to 14 days. These amounts were often greatly in excess of that found 24 hours after a fatty meal, and were apparently due to the mobilisation of depot fat. In fasted, enterectomised animals, the thoracic duct lymph was clear, and this was taken to indicate that the source of the milky lymph normally found during fasting was (by reabsorption) from the lumen of the bowel, the lipids having been previously secreted into the lumen, from the blood, by the mucous membrane. As a possible purpose of such a mechanism it was suggested that, just as, after fat feeding, the chyle fat had a different melting point and iodine number from that ingested (indicating some modification by the intestinal mucosa during absorption) similarly some of the mobilised fat was acted on by the intestinal mucosa before it could be utilised.

It is an opinion difficult to accept as it stands. However, on their theory, the major portion of the secreted lipids was hydrolysed in the lumen, reabsorbed by the intestine mucosa, resynthesised and passed into the lacteals.

One/

One further interesting theory was advanced. Johnson & Freeman (1938), in experimental work done on dogs, in which lymph was collected from the lacteals and the thoracic duct during active fat absorption, found a haemolytic agent present in such samples. They suggested that the products of fat digestion were absorbed via the lymphatic pathway, instead of directly to the blood stream, as a protection against haemolysis by this toxic agent. The haemolytic effect was minimal both early and late in the experiments, and maximal when the lacteals appeared most milky and fat absorption was at its height. The injurious substance, in its slow coursing through lymphatics and lymph nodes, tended to disappear, or was greatly diluted by lymph from other sources.

In further investigations (1940) they attributed this haemolysis to fatty acids and soaps found in the lymph in quantities ranging from 1 - 5 mg. per c.c.

Clinical and experimental observations on the effects of disease and resection of mesenteric nodes on the absorption of fat, seemed more to illustrate, however, that a purely mechanical obstruction resulted to the pathway of absorption, rather than that fat metabolism, etc., was normally subserved by the regional nodes. (Poynton & Paterson, 1914; Jones, 1924; Pratt/

Pratt & Frew, 1931; Fairley & Mackie, 1937; Hill, 1937; Glynn & Rosenheim, 1938; Klein & Porter, 1944; Clarke, Ivy & Goodman, 1948).

In summary of the present position - intracellular and extracellular sudanophilic material is found in the mesenteric lymph nodes during and immediately after the active absorption of fat. Phagocytes again laden with Sudan-stained droplets make their reappearance during starvation, apparently mobilising the depot fat.

Although it has been variously suggested that synthesis or breakdown of the products of fat absorption occurs in their passage through the nodes, the exact nature of the part played by the mesenteric lymph nodes in the phenomena of nutrition is still in the realm of conjecture.

Lymphoid tissue in general is sensitive to dietary influences, and marked atrophy results from malnutrition and especially from a dietary deficiency of fat. Mesenteric lymph nodes on the other hand are known to enlarge on a diet rich in fat.

II. THE SCOPE OF THE PRESENT THESIS.

This review of past work would not be complete without indicating the broader problems upon which the present study is based.

I. Sites of Absorption.

Most of the past histological work has been done, naturally enough, on the site of maximum absorption - the small intestine. Closer inspection reveals that the majority of investigations reported have been confined, in the main, to mid-jejunum.

While several investigations have been carried out to determine whether or not fat can be absorbed from the stomach, and fat droplets have been described in the epithelium lining this site in some animals, the question remains unsolved as to the nature of the absorbed substance and whether or not this can be regarded as true absorption in that it undergoes further transport.

The question also arises whether the long stay afforded fat in the stomach of certain animals results in lipolysis and absorption.

Similarly, it has been a general assumption, unsubstantiated largely by histological evidence, that most/

most fat has been absorbed by the time it reaches the caecum, and that none is absorbed there.

While there is indisputable evidence, histologically and experimentally, to indicate that some absorption of some fatty substances can occur from the large intestine, little has been done to determine the nature and extent of this, or to correlate it with the varying dietary tendencies and fundamental structural differences in the various parts of the colon in herbivorous, carnivorous and omnivorous types. It is to be expected, too, that some correlation exists between the histological picture and the observed facts that large amounts of lipids excreted by ileum are apparently reabsorbed in the colon. Fatty acids from cellulose fermentation in some animals are similarly so absorbed.

Some reference has been made by Frazer (1946) to simultaneous observations in duodenum and lower parts of the small intestine at stated intervals after the ingestion of fat, but little attempt has been made at a comparative study of this aspect of the problem. No comprehensive histological survey has been carried out which would determine the degree of absorption which, under reasonably physiological conditions, could/

could be seen to occur at different time intervals from each region of the gastro-intestinal tract in a series of animals of approximately the same age, but representing species with differing types of colon and of varying dietary habit.

Accordingly the present work seeks to trace and compare the fat absorption picture as seen histologically, at various absorptive phases throughout the entire length of such differing alimentary tracts.

2. Mucosal Stroma and The Migratory Cells.

It seems more or less established that fat droplets are discharged freely from the epithelial cells into the stroma of the villus, and eventually into the lacteal, without the intervention of an intermediate agency such as that of phagocytic cells.

Although it has been variously suggested that the migratory cells in the stroma of the mucosa and submucosa of much of the intestinal tract are lymphocytes, eosinophils, and phagocytic cells of the reticulo-endothelial system, even the most recent assessments show that evidence on the exact nature of the cells and of their granules is still inconclusive.

Only/

Only the direction of their migration seems certain, and even here it would seem that this may apply to only one type of cell. Their source of origin, their probable function, and whether or not, in their invasion of the intestinal wall, they show a periodicity related to digestion and the processes of absorption are matters still open to doubt.

It is also far from clear whether they are similar to, or differ from, those cells containing Sudan-stained inclusions which occur in the loose lymphatic (sinus) tissue of the intestinal lymph nodes during the active absorption of fat.

This survey seeks to ascertain the nature of these cells, to trace their source of origin, to confirm the direction of migration, and to determine whether or not a relationship can be detected between their appearance in the gut wall and the processes associated with digestion. In connection with this last point, their distribution as seen in frozen sections cut at approximately 10 μ thick, from each region examined, is described and discussed.

Since it seems uncertain whether or not phagocytic cells of the reticulo-endothelial type do in fact/

fact occur in the stroma of the villus or in the lamina propria of the mucosa of other regions of the gut wall, this question receives separate attention in the pages that follow.

3. Lymph Vessels of the Gut Wall.

Mucosal, submucosal, muscular and subserosal networks of lymphatics in the gut wall were described by Schafer (1912).

Apart from occasional references to fat stained material in random lymph vessels of one or other of these plexuses, very few studies comment on the presence or absence of such material in the lymph vessels at the varying levels of the same animal's gastro-intestinal tract at different absorptive periods, while it seems clear that such material, when present, is free and extracellular, the presence or absence of the so-called migratory cells in these vessels has neither been confirmed nor refuted.

Both these matters receive attention in the following study.

4. The Regional Lymph Nodes.

During and after absorption, sudanophilic material in the free state can be seen in the mesenteric lymph nodes. It is by no means certain, however, what changes, mechanical or chemical, affect it in its passage through the node.

While much of the fat is apparently phagocytosed by reticulo-endotheilai macrophages, the nature of the intracellular changes, if any, and indeed the form in which the fat leaves the node, are uncertain. Is all, or only part, of the absorbed fat phagocytosed? Does the fat leave the node, as it enters it, in the free state? If so, what purpose does the intervention of the macrophage cells subserve?

As has been suggested, these phagocytic cells have been tacitly equated by many workers, without it seems adequate justification, with the free cells seen invading the gut wall.

An attempt is made here to clarify this last point, and to describe and compare the histological appearances, as seen in Sudan-stained sections from intestinal lymph nodes taken at the various absorptive times, from each of the animals examined. Where it seemed/

seemed helpful, additional sections were stained by the Periodic-Acid-Schiff method.

III. ORIGINAL WORK.

1. FERRET.

FERRET.

CONTENTS.

FERRET.

MATERIALS AND METHODS.

FERRET.

In this comparative study of the histological appearances in the gastro-intestinal tract and its regional lymph nodes during fat absorption, the ferret was chosen as representing the carnivora.

Materials and Methods:

Animals: Two fully mature, piebald, male ferrets, weighing 1075 and 1185 grams respectively, and five 3 month-old ferrets (3 female & 2 male), ranging from 400 to 480 grams, were obtained from a local source. All were clean, well-fed and in good condition.

Diet & Dietary Habits: The young ferrets were weaned at three months, and thereafter, like the adults, were fed, twice daily, on a diet of bread and milk. Minced liver and horse-meat were occasionally added.

They were allowed to accommodate to their new surroundings, and except for an initial loss of appetite on the part of the adults, showed no sign of upset. All remained active and gained weight.

It was noted that when continuous access to food

was/

was given, the adults especially, tended to feed only in the early evening. Without exception, food was left untouched throughout the hours of the night. It is perhaps of relevance to point out, in this connection, that of all the species examined, in the ferret alone could the overnight fast preliminary to experimentation be regarded as truly 'physiological'.

In an endeavour to avoid both grossly abnormal dietary types and amounts of fat, it was decided for the purpose of these experiments, in common with those on other animals, to use cream skimmed from the top of whole milk, and hereafter referred to as 'skim-cream' (Thorpe, 1947), as the nearest available approach to a physiological source of fat. The fat content of such cream is approximately 15-20 per cent.

The use of 'skim-cream' had the additional advantage of containing both the water-soluble vitamin B and the fat-soluble vitamin A. In view of the experimental results of Mottram, Cramer & Drew (1922), this seemed perhaps important, since the effect of the lack of these vitamins on the fat absorption picture was found to be immediate.

'Skim-cream'/

'Skim-cream' differs from certain other fats in containing all saturated 'even carbon' fatty acids from butyric (C 4) to lignoceric (C 24), as well as a variety of unsaturated acids. "It has a greater proportion of fatty acids of low molecular weight than has any other natural source" (Thorpe, 1947).

In view of the recent biochemical work reviewed in the first chapter, this wide range would seem desirable to ensure optimum opportunities for absorption, by all possible sites and routes.

The appetite of the animals concerned was as far as possible taken as the gauge of the amounts to be used. It was found that the young ferrets would take eagerly 45-50 c.c. of 'skim-cream', and the adults from 50-70 c.c. Such amounts were consumed over the course of about half an hour.

Experimentation: At the end of 4 weeks the animals were fasted overnight - all food being removed from the cages from 5 p.m. to 9 a.m., and free access to water maintained.

One 4 month-old female ferret was killed in the fasting state.

The/

The remaining animals were given 45-50 c.c. of 'skim-cream'. All drank voluntarily. Thereafter one young ferret was killed at 1½, 3, 4½, and 6 hours. The adults were killed at 4 hours.

Each animal was weighed before fasting, feeding and again before death. There was invariably an overnight loss of weight which, in the circumstances, since they normally fast for this period, must be regarded as physiological. In the case of the young ferrets, which at the time of the experiment weighed from 650-770 grams, the fast accounted for a variable individual loss ranging from 50-100 grams, about half of which was readily made good within a few hours of the voluntary ingestion of 'skim-cream'. At the time of killing, therefore, all animals showed a definite rise in weight subsequent to the fall induced by the overnight fast.

Each animal remained active, and there was no evidence, at post-mortem, of any pathological lesions.

Lethal Agent: In all experiments Coal Gas was the lethal agent employed.

Tissues removed for Examination: The intestinal tract/

tract of the ferret showed several marked differences from that of the other species examined. These are discussed later in the section dealing with macroscopic features.

In view of these differences the regions chosen for histological examination could only approximate to those taken from other species. Accordingly, in addition to portions from the upper and lower thirds of the oesophagus, the fundus, body and pyloric end of stomach, the first part of duodenum, (immediately distal to pylorus) and the third part of duodenum; portions were taken at intervals of 15 to 20 cms. along the whole course of the intestine, the last specimens being taken 8 cms. and 4 cms. from its termination. Eleven portions were thus removed from the larger of the 2 adult animals, and six portions (A - F) from the second adult and from each of the young ferrets. (Ferret: Plate I). The cardiac portion of stomach was also examined from each of the young ferrets.

A large lymph node in the root of the mesentery was a constant finding and was removed for histological examination. Others occurred in close proximity/

proximity to the gut wall at intervals along its course. Those from the 2 terminal portions of intestine were removed with the appropriate piece of gut wall. One node related to the pyloro-duodenal junction was taken from the animals killed at 1½, 4½ and 6 hours.

Methods of Fixation: One adult ferret was fixed by aortic perfusion. A cannula was inserted to the aorta through the left ventricle of the heart and 1 litre of normal saline at approximately 40° C. followed by 1½ litres of 12% neutral formalin, at the same temperature, were perfused through the vascular system, drainage being permitted through an opening in the right side of the heart.

The abdominal cavities of the remaining 6 ferrets were opened immediately after death and the appropriate tissues removed, with the minimum amount of direct handling, to 12% neutral formalin for 24 hours.

Methods of Sectioning: Sections of approximately 7-10 μ in thickness were cut on the freezing microtome. (In Dextrin-saturated solution in water with a trace of phenol.)

Additional/

Additional material from each region of the 4 hour adult ferret fixed by aortic perfusion, and from the young ferret killed at 4½ hours, were paraffin embedded.

Between 20 and 30 sections from each region were examined histologically.

Methods of Staining: From each region taken for examination frozen sections were stained by each of the following methods.

Sudan IV (Gurr).

1. Sections were washed in distilled water - several changes.
2. 70% alcohol - 5 minutes.
3. Saturated solution of Sudan IV in 70% alcohol (filtered before use) - overnight.
4. 70% alcohol - 5 minutes.
5. Sections were again washed in water.
6. Haemalum counterstain - 1 minute.
7. Rinsed in water.
8. 'Blued' in water - 1 minute.
9. Sections were mounted in water media. (Kaiserling's Glycerine Jelly or Farrant's Medium).
10. Cover-slips were ringed with microscope cement of sealed off with paraffin wax.

Sudan Black (Gurr).

1.

Sudan Black (Gurr) Contd.

1. Sections washed in distilled water - several changes.
2. 70% alcohol - 5 minutes.
3. Saturated Solution Sudan Black in 70% alcohol (filtered before use) - 30 minutes.
4. 70% alcohol - 5 minutes.
5. Washed in water.
6. Haemalum counterstain - 1 minute.
7. Sections rinsed in water.
8. 'Blued' in water - 1 minute.
9. Sections were mounted as for Sudan IV.

Some sections from each region were examined without counterstain, and a few from the 4½ hour duodenum were stained by the Periodic-Acid-Schiff (P.A.S) Method. (See RAT - Materials and Methods).

Sections from the paraffin-embedded material were stained by the traditional Haemalum and Eosin method.

FERRET.

RESULTS.

FERRET.

Results.

Macroscopic Features:

The intestinal tract of the ferret showed several marked differences from that of the other species examined.

A much-elongated oesophagus entered the large, sac-like stomach lying high in the left, upper quadrant of the abdomen. This passed into a C-shaped duodenum, which was long relative to the total length of the gut. (The duodenum has been displaced in the diagram (Ferret, Plate 1) to facilitate demonstration of the other features).

Thereafter, it was impossible to distinguish macroscopically the various regions of the intestine. There was no marked variation in the diameter of the tube throughout its length, which, from pylorus to anus, in the fully mature animal, measured 186 cms. No recognisable caecum or other sharp transition from small to large intestine gave a clue to the latter's commencement. Several loops of bowel were suspended by a mesentery from the posterior abdominal wall. These passed,/

passed, with no perceptible change, at a right-angled hepatic flexure, into what appeared to be the equivalent of a short length of descending colon. This lay closely applied to the posterior abdominal wall, and terminated, after approximately 8 cms. at the recto-anal junction (Ferret, Plate I).

In those animals fed with milk or cream, the stomach at post-mortem, was distended with a soft, whitish-cream, sour-smelling, cheesy curd. A milky fluid exuded from the cut end of the intestine throughout much of its length. In the terminal 25-30 cms. the low-residue contents took the form of small, discrete, yellow, curd-like plaques, lying in a watery medium.

Stools from all ferrets were invariably watery, unformed, and contained little obvious roughage. This was true for those fed on milk and bread in the laboratory, and for those given supplementary liver and horse-meat, here or on local farms. It seemed probable, from naked-eye appearances, that there was a considerable prodigality in their utilisation of foodstuffs.

A large lymph node in the root of the mesentery was/

was a constant finding. Others occurred in close proximity to the gut wall at intervals along its course.

Microscopic Features:

Short notes on the main structural features of each region are incorporated in the following descriptions, the chief purpose of which, however, is to indicate the histological findings with relation to fat absorption, in sections taken from the fasting and various absorptive states, and stained with the fat-soluble dyes, Sudan IV and Sudan Black.

As will be seen, the principle feature of note in the ferret intestine is the presence of villi to within a few centimetres of its termination. Even this finding, however, is overshadowed in the younger ferrets by the discovery of sudanophilic droplets in the cells of the epithelium lining the last portion of the colon beyond the level at which the villi have ceased. In general, the sites involved in active absorption of fat, or its split products, appear to be much more extensive in the young ferret than in the other animals examined. This is perhaps not surprising, in view of the larger part played by fat in/

in their normal diet.

The crypt epithelium of duodenum, jejunum, etc. frequently showed two or three of its cells to be frankly basophilic. This feature is met with again in the guinea-pig, where the site strongly suggested that the cells concerned were the Paneth cells.

Oesophagus:

This organ was examined in its upper and lower thirds in each of the young ferrets.

It was lined throughout by stratified squamous epithelium of the keratinised type. Fatty material in the lumen adhered to the free surface of the upper third and sometimes obscured the histological picture. In spite of this, however, the Sudan stains appeared to have diffused into parts of the upper cornified layers, and were often present, even in the fasting animal, as minute globules . These latter, however, may have been carried in by the knife-edge in sectioning. (Ferret, Plate 2; Figure I). Slightly less Sudan-colouring was seen at 1½ and 3 hours (Ferret, Plate 2; Figure 2), but only minor fluctuations occurred throughout the ferret series. In all cases the stratum lucidum was coloured a pale orange with

Sudan/

Sudan IV, and blue-black with Sudan Black. With both stains it was highly refractile.

The granules of the stratum granulosum were sharply picked out by the counterstain. In many slides the serous caps of the mixed gland acini of the submucosa stained a dark purplish-red with Sudan IV, and a granular, greyish-black colour with Sudan Black. In no case, however, did they approximate in colour reaction to that found in the keratinised layers.

Much less Sudan-staining occurred in the lower than in the upper third. In the lower portion, only localised patches of Sudan-positive material were found at infrequent intervals near the luminal surface of the epithelium, and again no significant differences in the amount were noted in the fasting animal or in those killed 1½, 3, and 4½ hours after the administration of 'skim-cream'. The 6 hour sections were almost completely negative.

Stomach:

a) Adult Ferret.

Sections taken from fundus, body and pylorus of the adult male ferret stomach showed few unusual structural features. In the pylorus the mucosa was thicker,/

thicker, the gastric pits deeper, and the glands longer than in the other two sites. Several small isolated, lymphatic nodules in the mucosa lay entirely internal to the muscularis mucosae.

In frozen sections of formalin fixed tissues from the fully-grown male ferret, killed 4 hours after the voluntary ingestion of 50 c.c. of 'skim-cream', there was no evidence of sudanophilic material in the mucus-secreting, columnar epithelium lining all three sites.

Migratory cells, containing Sudan-stained granules, occupied the innermost region of the lamina propria, in the narrow zone between the muscularis mucosae and the base of the test-tube glands. Approximately two or three such cells were seen round the foot of each gland. (Ferret, Plate 3, Figure 3). They were increased in number in the vicinity of blood vessels, especially where these emerged from the submucosa, and were occasionally seen within their lumina. There was little evidence of further migration into the substance of the mucosa itself, except in the pyloric region, where scattered cells of the series occurred at intervals within the stroma, and in the widely dilated vessels which ran from the submucosa,/

submucosa, through the lamina propria, parallel to the test-tube glands and at right angles to the mucosa's free surface.

Lymph vessels at all levels of the wall failed to show any trace of the fat-soluble dye.

The gastric mucosa from a second full-grown, mature, male animal, 4 hours after the ingestion of cream whose tissues were fixed, immediately after death, by aortic perfusion of 12% neutral formalin, showed essentially the same picture.

In both animals the haemalum counterstain picked out the distinctly basophilic nuclei, and confirmed the impression that the granules of the migratory cells were the sudanophilic elements.

b) Young Ferret.

These findings did not accord entirely with those in the four-month-old ferret killed at a similar time-interval after feeding with 'skim-cream'. This animal, and its litter-mates, all of whom had been weaned for 4-6 weeks, were fasted overnight, and killed, either in the fasted condition, or 1½, 3, 4½ and 6 hours respectively, after feeding with 'skim-cream'. Sections/

Sections stained by Sudan methods gave slightly different pictures at the various absorptive stages.

In the cardiac portion of stomach, the mucus-secreting, columnar epithelium remained negative for fat throughout the series. The fundus and upper part of the body of stomach, at 3 and 4½ hours showed fine, dustlike granules of sudanophilic material in a few surface cells. These granules, which occupied both the supranuclear and the tapering, infranuclear portions of the cell, showed a much wider distribution in the epithelium lining the lower body of stomach and the pyloric region. Residual traces were seen in the fasting and 1½ hour specimens, (Ferret, Plate 4, Figure 6), and again in those taken at 6 hours, when the granules were predominantly infranuclear. At 3 and 4½ hours almost all lining cells in direct contact with the luminal contents showed a preponderance of granules above the nucleus, deep to the u-shaped, mucus-containing part of the cell. (Ferret, Plate 4, Figure 5).

There was no histological evidence to suggest further transport of this material to blood or lymph.

Few, if any, migratory cells were seen in any region/

region of the stomach wall in the fasting animal. These cells began making their appearance in the lamina propria near blood vessels, and around the base of the test-tube glands at 1½ hours. They were greatly increased in all areas, and indeed reached their maximal numbers in the cardiac area, at 3 hours, when occasional, additional members were found in the stroma and blood vessels of the submucosa and invading the substance of the mucosa itself. (Ferret. Plate 3, Figure 4). They were still present in considerable numbers in all sites at 4½ hours, but were at their greatest then in the lamina propria of the fundic and pyloric regions. Here again, as in the other sites, although still mainly confined, at 6 hours, to the base of the glands, they had diminished in number and become more irregular in distribution. At no point was there general invasion towards the luminal surface of the mucosa by these cells, but a few were seen to migrate into the mucosa in the fundic and pyloric areas at 4½ and 6 hours, and some were recognisable, and still distinctly sudanophilic, within the lumen of the stomach itself.

Results were substantially the same whether Sudan/

Sudan Black or Sudan IV was employed.

It is perhaps of significance to note that in frozen sections stained by both methods, the oxyntic cells showed faint tinting with the stains - greyish-black or pale orange, respectively.

Paraffin-embedded tissues, stained with haemalum and eosin, revealed the presence of polymorphonuclear leucocytes, many of them with horse-shoe or ring-shaped nuclei, in sites corresponding to those occupied by the sudanophilic granular cells in the frozen, Sudan-stained material. But while granules were distinct and positive with the latter stains, it was impossible with haemalum and eosin, to distinguish the integral, cytoplasmic granules which make sub-classification of granulocytes possible. Although their cytoplasm was frankly eosinophilic, no coarse granules of the eosinophil class were to be seen.

Duodenum:

a) Adult Ferret.

In the wall of the first part of the duodenum of the fully-grown, male ferret, although the striated border/

border had made its appearance, there was no visible absorption at 4 hours of fatty material into the cells of the epithelium or the vessels of the lymphatic networks.

Granulocytes, with Sudan-stained granules, crowded the stroma of the long, slender villi, some being seen in the lamina propria between adjacent crypts, and a few at the root of the mucosa. Approximately 25 to 30 of these cells were seen in the core of the average villus, but although they often came near to the surface, at no point were they in active migration between the cells of the epithelium.

Again, as in the stomach, a few were intravascular, and occasional members were visible near the blood vessels of the submucosa.

In the third part of the duodenum, below the entrance of the bile duct, the picture had undergone a dramatic change. Minute, sudanophilic globules filled the supranuclear parts of the cells covering most of the villi, while many dust-like granules had already reached the tapering, infranuclear zones. Larger globules about the size of the nucleus occupied many of the cells at the tips of the villi.

There/

There was little evidence in these sections of further passage to the stroma of the villi or the lumen of the central lacteals. Lymphatic vessels of the networks remained negative to the stain.

Granulocytes in even greater numbers flooded the stroma of the villi and between the crypts, and were especially prevalent in the upper half of the mucosal field. Again no evidence of active migration from stroma to gut lumen was found.

The picture in the adult animal fixed by aortic perfusion resembled that seen in the tissues prepared by ordinary methods of fixation.

Lymph vessels in the submucosa and intramuscular network of both parts of the duodenum, however, showed granular or homogeneous blue-black contents with Sudan Black in this animal. Central lacteals in the villi and between crypts of the third part were similarly stained.

b) Young Ferret.

In young ferrets (even in the fasting animal), the first part of the duodenum showed traces of sudanophilic granulations in the cells at the tips of

a/

a few villi. Minute droplets, chiefly supranuclear, were found in the cells covering entire villi at 1½ hours (Ferret. Plate 5, Figure 7). None of these drops, however, were seen in the act of passing through the striated border, which remained clear of the stain throughout the series. With Sudan IV, a fine orange granulation persisted in some cells, especially at the tips of the villi, in 3, 4½ and, to a decreasing extent, in 6 hour sections.

At 1½ hours, up to a dozen granulocytes were seen, chiefly within the core, of each villus. Occasionally such cells occurred in the submucosa, but none were demonstrated in the lymphatic nodules, which, together with Brunner's glands, disrupted the strands of the muscularis mucosae.

The numbers of granulocytes had increased markedly at 3 hours, when as many as 40 to 50 migratory cells crowded the stroma of each villus. Here again they were confined chiefly to the upper half of the mucosa but were occasionally seen in the tissue between crypts and in the submucosa. They were dwindling in numbers at 4½ hours, and had returned to the range of 6 to 12 per villus at 6 hours.

Although/

Although none were seen passing through the epithelium itself, debris within the lumen of the gut at 3, 4½ and 6 hours, showed clumps of recognisable leucocytes whose granules stained distinctly with Sudan IV and Sudan Black.

Only slight traces of extracellular, sudanophilic droplets were seen in the stroma of the villus, and these were mainly in the 1½ hour sections, in which several central lacteals in the villi and occasional lymph vessels between crypts, and in the submucosa, showed granular, Sudan-stained material and droplets of varying sizes. (This piece of tissue, together with the corresponding portions from the other animals, had been removed from the area immediately distal to the pylorus. The presence of Brunner's glands in the submucosa tended to confirm that it was indeed the first, and not the second, part of the duodenum).

In the younger, as in the adult animal, there was a striking change in the picture below the entrance of the bile duct.

In sections taken at 1½ hours the epithelial cells covering all villi were involved in active absorption of fat. Droplets of varying, but mainly small size crammed the supranuclear cytoplasm of

the cells, while very fine droplets already occupied the infranuclear zone, and had penetrated to the stroma of the villus. Central lacteals showed granular staining sometimes of almost homogeneous intensity. The continuity of these lymph vessels was occasionally traced between crypts and in the submucosa.

From 12 to 25 positive-staining granulocytes were confined almost exclusively to the core of each villus, a few being seen between crypts in the luminal half of the mucosa.

No marked change in the amount of fat-staining material was seen at 3 or 4½ hours. Staining in the epithelium was perhaps less intense at 3 hours, due to the more discrete, intracellular distribution of very fine particles, while at 4½ hours many intracellular globules, especially at the tips of the villi, were as much as 4 or 5 times the size of the nucleus. Lymphatics at the base of the mucosa were particularly prominent at 3 hours, while the granulocytes tended to congregate in the stroma of the root of the villus at 4½ hours.

Droplet inclusions, although not of uniform size,/

size, tended to be smaller at 6 hours, and not all villi were involved. Granulocytes, while of similar distribution, were much more numerous and were frequently seen between the cells of the epithelium and within the luminal debris.

The Intestine:

a) Adult Ferret.

Sections from the upper jejunum of the adult ferret killed at 4 hours resembled those taken from the third part of the duodenum. Villi were perhaps longer and the total mucosa thicker. Sudanophilic droplets were less densely distributed in the cells of the epithelium, and fewer villi were involved. Positive staining granulocytes, 40 or 50 of which associated with the stroma of each villus, were distributed as in duodenum.

Fifty centimetres from the arbitrary duodeno-jejunal junction, residual fatty material in the columnar cells covering some villi suggested that the full flood of absorption had passed. These sudanophilic inclusions were granular, and only in a few places densely stained. Up to 150 leucocytes with Sudan-stained integral granules crowded the lamina propria of each villus and were strung out between/

between adjacent crypts. These were undoubtedly present in larger numbers than any encountered in other regions.

Fifteen centimetres further on the picture was virtually unchanged. The fifth and sixth parts examined showed even less involvement of the lining epithelium, though enormous numbers of granulocytes still flooded the field.

Villi became progressively shorter and the wall of the gut much thinner. The epithelial cells were now entirely negative with the fat-soluble stains. Very pale orange or blue-black granulation was seen within the luman of a few submucosal lymphatic vessels, one of them running in a small lymphatic nodule.

Eventually, in the ninth part, though the striated border was retained, the villi had disappeared, and the greatly folded mucosa no longer held such great numbers of granulocytes. A few were seen in and around blood vessels or in the stroma especially at the base between crypts. Occasional members of the series were detected in irregular distribution in the stroma and blood vessels of the submucosa. A very faint orange pink cytoplasmic granularity was seen with Sudan IV in some of the epithelial cells. Even this

was missing from the tenth and eleventh parts which were taken from the colon as it lay against the posterior abdominal wall 8 and 4 centimetres respectively, from its termination.

Six portions (A - F) (the first four at approximately equidistant intervals, the last two from the terminal colon) were examined from the animal fixed by aortic perfusion. These showed essentially similar features to those already described.

Maximum involvement of the epithelium was again found in the upper jejunum (Portion A) (Ferret. Plate 7, Figure 11). Minute Sudanophilic granules were still present in the supranuclear position of the epithelial cells in the portion B. Orange or blue-black cytoplasmic granularity was, however, still seen in the lining cells of the fourth and subsequent sections (D - F).

Granular orange or blue-black staining material was present in central lacteals and submucosal lymphatic vessels in the first three portions of Intestine (A - C), and in the submucosal vessels of the Fourth (D).

Villi extended to within the last few centimetres of the intestine. In all sections, where these were present,/

present, enormous numbers of migratory cells with the customary Sudan-stained granules flooded the lamina propria, especially in its upper half. (Ferret. Plate 7, Figure 11; Plate 8, Figures 12 & 13; Plate 10, Figures 15 & 16). In the last two sections (E & F) - from the hepatic flexure and from the terminal 4 centimetres - they were fewer in number and confined mainly to the base of the mucosa, and to the blood vessels and stroma of the submucosa. Only scattered cells strayed to the tissue between crypts. (Ferret. Plate 10, Figure 16; Plate 11, Figures 17 & 18).

b) Young Ferret.

The corresponding regions examined in the four month old ferrets revealed the presence of villi in all but the last portion taken. Aggregations of small lymphatic nodules, such as are characteristic of Peyer's patches, were found in portions C,D, and E.

In the fasting animal fine residual droplets of Sudan-stained material were found in some of the surface cells covering several villi in the lower part of the jejunum (Portion B). They were seen also in portions C and D (which had Peyer's patches in their wall), and were especially prevalent in the supranuclear zone/

zone of the epithelial cells of the terminal colon. (Portion F) (Plate 12, Figure 19).

The stroma remained negative for positive-staining, extracellular material throughout the fasting gut. Central lacteals, however, showed the stain in portion B, while submucosal lymphatics were positive in B,C, and D. Vessels of the intramuscular network were visible and stained in portion C.

Forty to fifty granulocytes crowded the core of the individual villus and were seen between crypts and in the submucosa in all regions except the last two (E & F). (Ferret. Plate 6, Figure 8).

In the absorptive state, the upper jejunum (Portion A) showed extensive involvement of its epithelium at 1½, 3, and 4½ hours. (Ferret. Plate 6, Figure 9). At 3 hours, supranuclear and infranuclear droplets, though of varying sizes, were still much smaller than the cell nuclei. The inclusions tended to be largest immediately deep to the striated border and to become progressively smaller as they passed from luminal to basal margins of the cell. Only a few, in the cells at the tips of the villi, approximated in size to that of the nucleus. Many more were in/

in this size-range at 4½ hours. (Ferret. Plate 7, Figure 10). Not only was grading present in the individual cell but it was conspicuous in the villus itself where some droplets, near the tip, were 4 or 5 times the size of the nucleus, while those in the cells near the base were fine or granular. Though most villi were still involved at 6 hours, especially in their upper halves, less extensive staining was noted. Droplets were graded in size, as before, from above downwards, and from without inwards, some very large ones being situated near the tips of villi.

Maximum staining of the epithelium in this and the lower part of the jejunum (Portion B) was found at 4½ hours. This coincided with maximal involvement of the central and submucosal lymphatics, which were, however, stained in all but the fasting specimens. (Portion A). The stroma contained minute, extracellular droplets of Sudan-stained material.

Granular leucocytes were abundant in all sections from Portions A and B, and varied in number from 50 to 100 per villus. They were confined mainly to the core of the villus and the upper lamina propria between crypts. In all cases the granules of these cells stained darker with Sudan stains than the fat droplets in/

in the epithelial cells, stroma and lymphatic vessels. The cells were probably maximal in number at 4½ or 6 hours, but no great difference in number or distribution was noted at the various absorptive stages.

In Portion C, very slight traces of palely stained, residual, fatty materials were present in some epithelial cells especially in the infranuclear zone at 1½ hours. Occasional droplets were also found in the stroma of the tips of the villi and in occasional lymphatic vessels of the submucosa.

Three and 4½ hours after the ingestion of 'skim-cream' there was a considerable increase in the epithelial involvement, and the sudanophilic inclusions, though still fine, were found immediately above the nucleus as well as in the infranuclear position. (Ferret. Plate 9, Figure 14). These droplets were found in the epithelial cells covering not only the villi but in those overlying the lymphatic nodules of the Peyer's patch. Again, as in the portions A and B, there was functional independence of the villi, with grading of droplet size from above downwards, etc., as previously described.

Free, sudanophilic droplets were seen in the stroma,/

stroma, in the central lacteals and in submucosal lymphatics which presented their maximal involvement at 4½ hours.

There was less Sudan-stained material in epithelium, stroma and lymphatics after 6 hours.

Migratory granulocytes, with their characteristic, brownish-yellow granules in Sudan IV sections, averaged 40-50 per villus at 1½ and 3 hours (Ferret. Plate 9, Figure 14), and were more widely distributed throughout the whole lamina propria at 4½ and 6 hours. These cells were conspicuously absent from all lymphatic nodules.

In Portion D of the intestine, villi were still elongated and typical of ileum. The only satisfactory material obtained from this area showed, at 3 hours, minute, but very distinct, sudanophilic granules in the supranuclear zone of the epithelial cells covering many villi. Droplets in stroma and lymphatic vessels of the villus and the submucosa also took up the stain. Migratory cells were present in the order of 100-150 per villus and its associated lamina propria. Some of these were clearly seen in the act of migrating through the epithelium. Lymphatic vessels/

vessels were very prominently displayed at 4½ hours, though only faint granular traces of sudanophilic material remained visible in the intestinal epithelium.

Eight to nine centimetres from the termination of the intestine (Portion E), the villi had become short and stumpy, and small groups of lymphatic nodules in the submucosa still disrupted the muscularis mucosae to enter the mucosal coat where they were covered on their upper surface by the intestinal epithelium.

The epithelium lining the gut at this point, which was negative in the fasting animal, held fine sudanophilic granules, chiefly in the supranuclear zone of the cells, 1½ hours after the ingestion of cream. These fine dust-like granules were very distinct and positive in all surface lining cells, but were absent from crypt epithelium, at 3 hours. They were very greatly increased in size and number at 4½ hours and were also found in the epithelium covering the lymphatic nodules. They now occupied the supranuclear and infranuclear zones of the cell, and, though still minute included some of larger size than any seen at 3 hours. There was still widespread epithelial involvement at 6 hours, but maximal Sudan-staining was seen at/

at 4½ hours.

Lymphatic vessels of the submucosa were negative at 1½ and 3 hours, but showed traces of positive staining material at 4½ and 6 hours.

Migratory cells, with the typical yellow-orange granules in Sudan IV, were very much fewer in number in this region of the gut. They averaged 6 per villus in the sections taken at 1½ hours; 2 to 3 per villus at 3 hours; 6 to 12 at 4½ hours; and up to 30 per villus in the later sections.

In the terminal colon (Portion F) the surface epithelium of the much folded but non-villous mucosa showed very many minute, sudanophilic droplets and granules in the supranuclear position of the cells. This very distinct Sudan-stained "dust", while present in the fasting, 1½ and 3 hour sections, was markedly increased at 4½ and 6 hours. (Ferret. Plate 12, Figures 19-21). In these latter sections, all cells in contact with the luminal contents of the intestine were involved. Droplets of varying sizes packed the supranuclear zones. Faintly positive granulation could be detected in the infranuclear position in the 3 hour and subsequent sections.

Submucosal/

Submucosal lymphatic vessels were seen to contain fatty material only in the 1½ and 3 hour sections. The lamina propria showed no extracellular, sudanophilic substance, and migratory cells of the granulocyte class were very few in number, 2 or 3 being the maximal number seen between any two crypts at 3 hours. A very few were found in the submucosa, especially where this was projected upwards into a fold of the mucosa.

The Regional Lymph Nodes of the Intestine during Fat Absorption:-

As indicated in Plate 1, a large lymph node in the root of the mesentery appeared to drain most of the intestinal tract. In addition to this large regional node, small, discrete nodes were found in close association with the wall of the gut in the region of the pyloro-duodenal junction, the hepatic flexure and the terminal colon. These nodes were taken with the appropriate portion of gut wall for histological examination.

a) Mesenteric Lymph Node:

In the 4 hour, post-ingestive, adult animal, the lymph node from the root of the mesentery showed traces of homogeneous, sudanophilic lymph in its network of loose lymphatic tissue. Many migratory cells containing Sudan-stained granules were found in this tissue. These were in all respects (appearance, size and staining reaction) similar to those free cells described as granulocytes in the gut wall. They were often most profuse in number in the vicinity of small muscular arteries. Several small groups of larger cells, the detail of which was completely masked by Sudan-positive droplets of varying sizes in/

in their cytoplasm, were found chiefly in the loose lymphatic tissue. These large cells appeared to be macrophages of the reticulo-endothelial system. (Ferret. Plate 14, Figure 24).

In the animal fixed by aortic perfusion, the subcapsular, interfollicular, and medullary sinuses contained abundant granular, strongly sudanophilic lymph. Cells of the granulocyte series were again scattered throughout the loose lymphatic or sinus tissue. Occasional large, phagocytic cells of irregular shape and with oval nuclei were also noted. These cells, which were three or four times larger than the granulocytes, contained sudanophilic droplets of varying sizes and were found in the loose lymphatic tissue. In addition, a faint orange tint could be seen with Sudan IV in the cytoplasm of fixed reticulo-endothelial cells of the germinal centres and of the loose lymphatic tissue, especially the subcapsular sinus. (Ferret. Plate 13, Figures 22 & 23).

In paraffin-embedded material, sections stained with Haemalum and Eosin showed similarly situated, free and fixed cells of the reticulo-endothelial system, with foamy, eosinophilic cytoplasm strongly suggesting the previous dissolution of fatty droplets during the process of fixation and embedding. Polymorphonuclear neutrophils/

neutrophils of similar size, distribution and nuclear arrangement to the cells described as granulocytes in the frozen material, were scattered throughout the loose lymphatic tissue.

In the young, fasting ferret, pale, Sudan-stained lymph was seen in many sinuses throughout the node. Afferent lymphatic vessels also showed this granular, free, positive-staining material. Considerable numbers of granulocytes took up the stain in the loose lymphatic tissue especially of the medullary zone. With Sudan IV, pale orange granulation was visible in the cytoplasm of some groups of fixed reticulo-endothelial cells in germinal centres and loose lymphatic tissue. Several, large, oval macrophages with oval nuclei and positive-staining droplets were found in the inner medullary zone. It is of importance to note that the "soft" orange tint of the droplet inclusions of both free and fixed macrophages was identical in all instances with that of the extracellular, Sudan-stained lymph of the sinuses, while the yellow-brown tint of the granules of the migratory leucocytes had a "brittle", highly-refractile quality.

One and a half and 3 hours after the ingestion of/

of 'skim-cream', localised areas of the subcapsular and interfollicular sinuses in the cortex, and the loose lymphatic tissue of the outer medulla, showed Sudan-stained lymph. After 4½ and 6 hours, traces of sudanophilic material still adhered to the network of the subcapsular sinus, but considerable amounts were now seen in the inner sinuses of the cortex and especially in those at the junction of cortex and medulla.

No free sudanophilic material was detected at any time in the dense lymphatic tissue of the nodules or medullary cords.

The coarse reticulum and the cytoplasm of fixed reticulo-endothelial cells in the germinal centre of many lymphatic follicles took a faint, granular, orange tinting with Sudan IV. This was a very much paler staining reaction than that of the free Sudan-stained material of the lymph.

Large, free macrophages of the reticulo-endothelial system were found in the loose lymphatic tissue occasionally in subcapsular sinuses but chiefly in the medullary zone. These large, phagocytic cells of irregular or oval shape, and with large, clear, oval or rounded nuclei, were filled with minute, strongly-/

strongly-sudanophilic globules of varying but mainly small size. They were more numerous here than in the fasting animal, and indeed much of the Sudan-stained material which in low power view had seemed to flood the medullary sinuses was seen, on high power examination, to be contained in the cytoplasm of great numbers of these cells. Very little free, extracellular material was found in the sinuses of the inner medulla and hilum. At 1½ hours many of the cells were filled with positive-staining globules to the point of masking the cell detail; others contained only a fine dusting of fine, discrete sudanophilic granules; yet others were almost completely negative. These cells, abundant at 1½ hours, flooded the loose lymphatic tissue of the inner medulla at 3 and 4½ hours, but, although similarly distributed, were fewer in number at 6 hours.

The over all impression gained was that free, extracellular, Sudan-stained material was perhaps maximal in amount at 3 hours, and was confined chiefly to the sinuses of the cortex and outer medulla. This could not be quantitatively determined, however, by histological examination alone, since much depended on the individual plane of section by which the relative amounts/

amounts of medullary and cortical tissue made available for study, varied.

Similarly, the free, phagocytic cells appeared to predominate in number at 3 and 4½ hours, but whereas those of the earlier sections showed pronounced individual differences in degree of involvement with fat droplets, maximal loading of individual cells was probably achieved at 4½ hours, the cytoplasm of almost all such cells being filled to capacity at this point. At 6 hours, on the other hand, the cellular inclusions were much more uniformly fine and dust-like.

Occasional migratory leucocytes, with their highly-refractile, orange-brown granules after Sudan IV, were scattered irregularly throughout the loose lymphatic tissue at 1½ hours. Very few were seen at 3 and 4½ hours, but considerable numbers were found in the sinus networks of the medulla in sections taken at 6 hours. They were thus probably maximal in number towards the end of the absorptive period studied. Their distribution, however, was so irregular that it was difficult to assess with any degree of accuracy their relative proportions, and it seemed likely that their numbers and distribution were uninfluenced by the stage of digestion and absorption.

Unfortunately/

Unfortunately, none of the sections examined showed the efferent lymph vessel, and it was thus impossible to ascertain the form in which absorbed fat entered the outgoing lymph stream.

b) Lymph Node from the wall of Portion E of Intestine.

Examination of the node found in proximity to the "hepatic flexure" (i.e. 8-9 centimetres from the colon's termination), from the fasting animal, revealed, in the medullary tissue, considerable numbers of free reticulo-endothelial cells with fine, sudanophilic granules in their cytoplasm. (Ferret. Plate 16, Figure 27).

Free, extracellular, strongly Sudan-stained material made its appearance in some of the subcapsular and interfollicular sinuses of the cortex and outer medulla in the 3 hour sections, while many of the large, oval macrophages with oval, pale, clear nuclei, flooded the inner medulla and held fine, positive-staining granules in their cytoplasm. These cells were again maximal in number in this site at 4½ hours (Ferret. Plate 16, Figure 28), and though fewer in number, persisted at 6 hours.

Granulocytes were again found especially near blood/

blood vessels.

Much more free material had penetrated to the inner medulla in the 4½ hour sections without being taken up by macrophages than was noted at any period in the mesenteric lymph node. Here both extracellular and intracellular sudanophilic material were abundant, and seen together, in the loose lymphatic tissue of the inner medulla.

Granular deposits were again seen in the reticulum of the germinal centres.

Positive-staining macrophages were similarly distributed in the medulla of the adult animal at 4 hours, but less free material was noted.

c) Lymph Node from wall of Terminal Colon:

From the fasting animal, the lymph node in close proximity to the wall of the terminal colon (i.e. 3-4 centimetres from its termination) showed only the faintest trace of sudanophilic material in the cytoplasm of some reticulo-endothelial cells of the subcapsular sinus. A few small groups of 3 or 4 large rounded or oval cells, chiefly in the medullary sinus tissue, contained positive-staining globules of unequal sizes which completely masked the nucleus. Only occasional, scattered granulocytes were noted, and/

and these were mainly in the loose lymphatic tissue near the wall of blood vessels.

The picture was essentially unchanged at 1½ hours. The phagocytic cells were increased at 3 hours and a few germinal centres showed positive granules. No evidence was seen of any free, extracellular, sudanophilic material in any of these sections, but a pale orange coagulum was noted with Sudan IV in one part of the subcapsular sinus at 4½ hours.

The corresponding node from the adult ferret fixed by aortic perfusion was virtually negative.

d) Pyloro-duodenal lymph node:

The picture in this node is of less significance with regard to determining sites of absorption, etc., than the other nodes examined, since the area of its drainage is uncertain.

Illustrations, however, are presented of its histological appearance, in the younger ferrets, 1½ and 4½ hours after 'skim-cream'. (Ferret. Plate 15, Figures, 25 & 26).

Many macrophages with sudanophilic inclusions were seen, but little or no free Sudan-stained lymph in the sinuses of the medulla.

This/

This node was not examined in the adult ferrets.

FERRET.

TABLES OF RESULTS.

FERRET.

Tables.

The histological findings during fat absorption in the ferret gastro-intestinal tract and regional lymph nodes are summarised in the following tables.

Unless otherwise stated, only those features which stain positively with the Sudan stains are incorporated. This includes the cells listed under Reticulo-Endothelial Cells in the regional lymph nodes. Only those containing sudanophilic inclusions are listed here.

The 'Epithelium' refers to that lining the tract in each area of the gut examined.

The 'Stroma' refers to that of the mucosa only, and a recorded negative result under this, or the 'Lymph Vessel' heading, implies that no extracellular or intracellular sudanophilic material, always excepting that seen in the migratory cells which are recorded separately, is to be seen in the stroma of the mucosa, or the lymph vessels at any level in the wall.

The/

The 'Migratory Cells' are granular leucocytes, and the sudanophilic elements in them are the granules. Only those cells picked out in this way by the Sudan stains are recorded. Some indication is given of their distribution and of the relative numbers seen in each site. Where actual numbers are given, these refer to cells counted in sections cut at 7 to 10 μ and are intended only as approximations to enable some comparison to be made of the degree of mucosal invasion by these cells at the various absorptive stages.

The cells listed under 'Migratory Granulocytes' in the descriptions of the Regional Lymph Nodes are in all respects identical with those seen in the gut wall.

The Time headings are self-explanatory.

Other Abbreviations adopted are mainly those in common usage, and include:

b.vs. - blood vessels.
l.vs. - lymph vessels.
Sud. - sudanophilic.

Terminology and Synonyms: The terms 'sudanophilic', 'Sudan-stained' and 'Sudan-positive' are used synonymously,/

synonymously, as are 'globule' and 'droplet'. 'Granule' or 'granulation', however, implies a much finer dispersion, or deposition, of the absorbed fatty material. Where the term 'Granularity' is used this denotes the microscopic appearance of the cytoplasm in question, and does not imply the presence of infiltrative sudanophilic inclusions.

'Loose lymphatic tissue' is used to denote the sinus tissue, cortical or medullary, of the lymph nodes.

Throughout the thesis the following descriptive words are used in terms of Pearse's definitions (1953).

'Lipid' or 'Lipide' - this is used to cover the whole of the large group of naturally occurring fat-like substances which are insoluble in such solvents as benzene, etc.
Under this heading are included the fatty acids which can be derived from simple and compound lipids by hydrolysis; the sterols (e.g. cholesterol); and phosphatides.

'Fat' - unless obviously applied to the dietary fat, is used to denote 'neutral fat'.

'Lipoid' - This term has been avoided as much as possible in the statement of results and in discussion.

It has been employed where quoting from the older writers, and here, as in the Discussion, it has been used to cover fatty substances in general.

FERRET : TABLE 1.

Adult Ferret : 4 hours after ingestion of cream:

	Epithelium.	Lymph Vessels.	Migratory Cells. (with Sudan-stained granules).
Stomach:			
Fundus	-	-	2-3 round base each test-tube gland. (Plate 3, Figure 3).
Body	-	-	Increased numbers near and in b.vs. of mucosa & submucosa.
Pylorus	-	-	(Pylorus- Also a few throughout mucosa).
Duodenum:			
1st.Part	-	-	25-30 in core each villus. A few in b.vs.
3rd.Part	Minute, Sud.+ globules in supranuclear zones; most villi. Also Infranuc. Granules. Larger globules at tips of villi.	Central lacteals; Submuc.; intramusc.	Increased nos.flood stroma villi & between crypts.
Intestine Portion A			40-50 per villus. (Plate 7, Figure 11)
Portion B	Minute, supra-nuclear inclus-ions.	Central lacteal; submuc.l.vs.	Up to 150 per villus. (Maximal nos.encoun-tered). (Plate 8, Figure 12).
Portion C	Less epithelial involvement.	As above.	Great nos. still flood field. (Plate 8, Figure 13).
Portion D	(Sud.granularity in Aortic Perfus. material).	Pale, Sud.+ granularity in submuc. l.vs.	As above. (Plate 10, Figure 15).
Portion E	As for D. (Villi persist)	-	Only a few, especial-ly near b.vs. or at base crypts. Some invasion of mucosa. (Plate 10, Figure 16)
Portion F	As for D. (but no villi)	-	Fewer than in E. (Plate 11, Figures 17 & 18).

FERRET : TABLE 2.

Young Ferret:-

Oesophagus : (Upper Third. Stratified Squamous Epithelium - keratinised).

	Epithelium.	Stroma.	Lymph Vessels.	Migratory Cells.
Fasting:	Some Sudan-staining in cornified layers. (Plate 2,Figure 1)	-	-	-
1½ hours:	Slightly less Sudan-staining. (Plate 2,Figure 2)	-	-	(Occasional granulocytes deep to basement membrane)
3 hours:	As at 1½ hours.	-	-	-
4½ hours:	As above.	-	-	-
6 hours:	As above.	-	-	-

N.B. Stratum Lucidum colours a pale orange with Sudan IV, and blue-black with Sudan Black. It is highly refractile with both stains.

FERRET : TABLE 3.

Young Ferret:

Oesophagus :(Lower Third. Stratified Squamous Epithelium - keratinised).

	Epithelium	Stroma	Lymph Vessels	Migratory Cells.
Fasting:	Only localised patches of Sudan-positive material at infrequent intervals near luminal surface.	-	-	-
1½ Hours:	As above.	-	-	-
3 Hours:	No significant differences in amounts seen.	-	-	-
4½ Hours:	As above.	-	-	-
6 Hours:	-	-	-	-

FERRET : TABLE 4.

Young Ferret : Stomach - Fundus:

	Epithelium.	Stroma.	Lymph Vessels.	Migratory Cells.
Fasting:	-	-	-	-
1½ hours:	-	-	-	Several in lamina propria near b.vs., and around base test-tube glands.
3 hours:	Fine, dust-like granules, sudanophilic, in a few surface cells. (Supranuclear & infranuclear.)	-	-	Great increase in numbers. (As in Cardia shown on - (Plate 3, Figure 4).
4½ hours:	As at 3 hours.	-	-	Maximal numbers. Same sites. A few migrating towards surface. Some in lumen.
6 hours:	-	-	-	Diminished numbers. More irregular distribution. Some in lumen.

FERRET : TABLE 5.

Young Ferret : Stomach - Body:

	Epithelium.	Stroma.	Lymph Vessels.	Migratory Cells.
Fasting:	Trace +	-	-	-
1½ hours:	Fine, sudanophilic granules in infranuclear position surface, mucus-secreting columnar cells. (Plate 4, Figure 6)	-	-	Several in lamina propria near b.vs., and round base of test-tube glands.
3 hours:	Fine, dust-like granules sudanophilic material in surface cells. (Supranuclear & infranuclear). (Plate 4, Figure 5)	-	-	Great increase in numbers; same site.
4½ hours:	As at 3 hours.	-	-	Still mainly confined to base of glands. Maximal numbers. No general invasion of mucosa seen, but some in lumen itself.
6 hours:	Trace +	-	-	Same sites. Numbers decreasing.

FERRET : TABLE 6.

Young Ferret : Stomach - Pyloric Region:

	Epithelium.	Stroma.	Lymph Vessels.	Migratory Cells.
Fasting:	Sudanophilic granules, chiefly infra-nuclear, in a few surface cells.	-	-	-
1½ hours:	As in fasting animal.	-	-	A few in lamina propria near b.vs., and round base of test-tube glands.
3 hours:	Almost all lining cells showed positive granules deep to u-shaped, mucus containing part of cell.	-	-	Great increase in numbers.
4½ hours:	As at 3 hours.	-	-	Maximal numbers. Several migrating towards surface; some in lumen.
6 hours:	Residual traces in lining cells.	-	-	Similar distribution. Diminished numbers.

FERRET : TABLE 7.

Young Ferret : Duodenum - First Part: (Above entrance bile duct.)

	Epithelium.	Stroma.	Lymph Vessels.	Migratory Cells.
Fasting:	Traces, sudanophilic granulation, cells at tip.	-	-	-
1½ hours:	Minute, supranuclear, sudanophilic droplets in cells covering entire villi. (Plate 5, Figure 7).	Slight trace free sud.+ material.	Central lacteal + Submuc.+	Up to 1½ dozen within the core of each villus. Occasional members in submucosa. (None in lymphatic nodules of submucosa.)
3 hours:	Very fine sudanophilic, supranuclear granulation in some cells, especially at tips of villi.	-	-	Up to 40 or 50 per villus. Occasional members between crypts & in submucosa. Some in luminal debris.
4½ hours:	As above.	-	-	Numbers diminishing.
6 hours:	Sudanophilic granulation persisting (but to decreasing extent) especially at tips of villi.	-	-	6-12 per villus. Some in luminal debris.

FERRET : TABLE 8.

Young Ferret : Duodenum - Third Part: (Below Entrance of bile duct.)

	Epithelium.	Stroma.	Lymph Vessels.	Migratory Cells.
Fasting:	-	-	-	-
1½ hours:	All epith. cells involved in active absorption of fat. Small drops in supranuc.zone. Finer particles deep in nucleus.	Fine droplets in stroma.	Central lacteals showed granular staining. Traced between crypts.	12-25 in core of each villus.
3 hours:	Fine droplets in most cells.	As above but amount increased.	Lymph vessels at base mucosa especially prominent.	As above.
4½ hours:	Maximal epithelial involvement. Many droplets, especially at tips villi, much larger than nucleus.	-"-	Negative	No marked change in numbers, but distribution chiefly in stroma at root of villus.
6 hours:	Droplet inclusions tended to be smaller in no. & size. Not all villi now involved.	A few droplets.	-"-	Similar distribution, but greater numbers. Frequently seen between epithelial cells & in lumen.

FERRET : TABLE 9.

Young Ferret : Intestine - Portion A: (Upper Jejunum).

	Epithelium.	Stroma.	Lymph Vessels.	Migratory Cells.
Fasting:	-	-	-	40-50 per villus. (Plate 6, Figure 8)
1½ hours:	Extensive involvement. Fine, sudanophilic droplets; supra- & infranuclear (Plate 6, Figure 9)	Minute, free droplets sudan-stained in core villus.	Central lacteals++ Submuc.++	As above. Confined mainly to core of villus, and lamina propria between upper part of crypts.
3 hours:	Droplets increasing in number & size. Largest deep to striated border,& at tips of villi.	As at 1½ hours.	Central lacteals++ Submuc.++	No significant change in number or distribution.
4½ hours:	Increasing size of droplet, with some at tips of villi many times larger than nucleus. (Plate 7,Figure 10) (Maximal epith. involvement).	As above.	Maximal involvement of central & submucosal lymphatics.	As above.
6 hours:	Most villi still involved, but less extensive staining present. Many large droplets still seen.	Free drops still seen.	Some staining in central & submucosal l.vs.	As above.

FERRET : TABLE 10.

Young Ferret : Intestine - Portion B: (Lower Jejunum).

	Epithelium.	Stroma.	Lymph Vessels.	Migratory Cells.
Fasting:	Fine, dust-like, residual droplets immediately above nuclei, in some cells lining some villi.	-	Pale but distinct sudan-staining in several central & submuc. vessels.	Large numbers throughout stroma of mucosa.
1½ hours:	As above.	Fine free droplets.	As above.	-"-
3 hours:	Marked increase in involvement. Droplets increasing in size & no.	As above.	Increase in staining reaction in central & submuc. vessels.	-"-
4½ hours:	Maximal epithelial involvement, & droplet size.	As above.	Maximal involvement of these vessels.	-"-
6 hours:	Inclusions much finer.	Fine droplets still present.		-"-

FERRET : TABLE 11.

Young Ferret : Intestine - Portion C: (Villi and Peyer's Patch present).

	Epithelium.	Stroma.	Lymph Vessels.	Migratory Cells.
Fasting:	Minute, dust-like droplets some cells, some villi. Chiefly above nucleus.	-	Submuc.+ Intramusc.+	40-50 per villus (Also occasional members in submuc.)
1½ hours:	Slight traces some cells; chiefly infra-nuclear. Pale sudan+reaction.	A few droplets in stroma, tips of villi.	Occasional submucosal lymph vessel.+	As above.
3 hours:	Minute, sudano-philic, supra-nuclear & infra-nuclear granules. Present in considerable amounts. (Plate 9,Figure 14)	Increase in free droplets present.	Central Lacteal++ Submuc.++	As above.
4½ hours:	Increase in epithelial involvement. Droplets still fine. Found in cells covering lymphatic nodules of Peyer's Patch.	As at 3 hours.	As above. (maximal involvement at 4½ hours).	More widely distributed throughout the whole lamina propria.
6 hours:	Slightly less epithelial involvement.	-	Slight trace in submuc.	As at 4½ hours.

(N.B. No migratory cells or free droplets of fat in lymphatic nodules).

FERRET : TABLE 12.

Young Ferret : Intestine - Portion D: (Villi present. Peyer's Patch).

	Epithelium.	Stroma.	Lymph Vessels.	Migratory Cells.
Fasting:	+ Granulation especially in infranuclear zone cells.	-	Submucosal vessel +.	40-50 per villus & associated lamina propria between crypts. Some in submucosa & in luminal debris.
1½ hours:	No satisfactory material obtained.			
3 hours:	Minute, supranuclear, sudanophilic droplets in many cells of many villi.	Free droplets in stroma of some villi.	Central Lacteal + Submucosal vessels +	100-150 per villus. Some in act of migration through epithelium.
4½ hours:	Faint, positive granulation.	-	Central Lacteal ++ Submucosal vessels ++	As at 3 hours. A few in submucosa.
6 hours:	No satisfactory material obtained.			

FERRET : TABLE 13.

Young Ferret : Intestine - Portion E: (7 cms. from termination. Villi present. Peyer's Patches.)

	Epithelium.	Stroma.	Lymph Vessels.	Migratory Cells.
Fasting:	Negative	-	-	-
1½ hours:	Minute, supranuclear, sudanophilic granules in many cells.	-	-	About 6 per villus.
3 hours:	All surface cells involved. (minute supra-nuclear granules)	-	-	2 or 3 in stroma of each villus.
4½ hours:	Droplets increased in size & number. (Maximal epithelial involvement). Supra- & infra-nuclear. Droplets also found in surface epithelium covering lymphatic follicles of Peyer's Patch.	-	Traces + submuc. vessels.	6-12 per villus.
6 hours:	Considerable involvement, but less than at 4½ hours. Droplets also found in surface epithelium covering lymphatic follicles of Peyer's Patch.	-	Traces + in submuc. vessels.	Up to 30 per villus.

FERRET : TABLE 14.

Young Ferret : Intestine Portion F: (Terminal Colon: no villi)

	Epithelium.	Stroma.	Lymph Vessels.	Migratory Cells.
Fasting:	Minute, Supranuclear, Sudanophilic droplets in many surface cells. (Plate 12, Figure 19)	-	-	-
1½ hours:	As above. (Plate 12, Figure 20)	-	Submucosal vessels showed sud. material.	A very few in lamina propria.
3 hours:	As above. Some faintly positive, infra-nuclear granulation.	-	As at 1½ hours.	2 or 3 in lamina propria between adjacent crypts.
4½ hours:	Larger droplets in all surface cells. Infranuclear granulation. (Maximal epithelial involvement). (Plate 12, Figure 21)	-	-	Very occasional, in mucosa & submucosa.
6 hours:	Considerable involvement, but less than at 4½ hours.	-	-	As at 4½ hours.

FERRET : TABLE 15.

Adult Ferret : 4 hours after 'skim-cream':

	Sudan Stained Lymph	Reticulo-Endothelial Cells.	Migratory Granulocytes.
Mesenteric Lymph Node	Some free material in parts of all sinus tissue. (Plate 13, Figures 22-23)	Several groups of free, oval or angular macrophages - detail masked by Sudan-stained droplets of varying size - in sinus tissue. (Several times larger than granulocytes). Occasional fixed R.E.cells Sudan-tinted in germinal centres. (Plate 14, Figure 24).	Many with sud. granules - in sinus tissue. (Similar to those described as migratory cells in gut wall). Most profuse in number near wall of small arteries.
Intestine-Portion E - Lymph Node	Less free material noted in adult than in young ferret at 4½ hours.	Sud.stained macrophages distributed throughout medullary sinuses.	Scattered cells.
Intestine-Portion F-Lymph Node	-	Virtually negative.	-

FERRET : TABLE 16.

Young Ferret:
Mesenteric Lymph Node:

	Sudan-Stained Lymph	Reticulo-Endothelial Cells.	Migratory Granulocytes.
Fasting:	Pale, sudan-stained lymph in many sinuses. Afferent l.vs. also showed granular, free sudan-stained material.	Some fixed R.E. cells of sinuses and germinal centres tinted with Sudan IV. Several large oval, free, macrophages, with Sudan-stained granules - inner medullary zone. (Inclusions distinct orange with Sud.IV).	Considerable numbers in sinuses especially of medulla, (yellow-brown granules with Sudan IV.)
1½ hours:	Localised areas of subcapsular, internodular, & medullary sinuses were positive.	Reticulum & fixed R.E.cells faintly tinted with Sudan IV. Large, free macrophages abundant in sinus tissue-occasionally in subcapsular, but chiefly in medullary zone. These cells filled with strongly Sud. gkobules of varying but small size. N.B. Some cells filled to capacity; others with only fine 'dusting' of granules.	Occasional cells scattered irregularly in sinus tissue.

FERRET : TABLE 17.

Young Ferret : Mesenteric Lymph Node:

	Sudan-Stained Lymph	Reticulo-Endothelial Cells.	Migratory Granulocytes.
3 hours:	Maximal amounts of free, extracellular sud. material, but confined chiefly to sinuses of cortex and outer medulla. Very little free sud. material in inner medullary sinuses.	These free cells, abundant at 1½ hours, flooded sinus tissue of inner medulla at 3 and 4½ hours.	Very few granulocytes seen at 3 hours.
4½ hours:	Still traces in parts of subcapsular sinus. Considerable amounts in inner sinuses of cortex and those at corticomedullary junction.	As at 3 hours. (Maximal loading of the greatest number of individual cells seen at 4½ hours.)	As at 3 hours.
6 hours:	As above.	Similarly distributed, but fewer in number. (Sud. inclusions finer & of more uniform size.)	Probably maximal in number now. Chiefly in medullary sinuses, but still irregular in distribution.

FERRET : TABLE 18.

Young Ferret : Lymph node from wall of Intestine(Portion E): (8-9 cms. from colon's termination)

	Sudan-Stained Lymph	Reticulo-Endothelial Cells.	Migratory Granulocytes.
Fasting:	-	Considerable numbers free R.E.macrophages in medullary tissue, showing fine, sud. granules in cytoplasm. (Plate 16,Figure 27)	Granulocytes were scattered irregularly throughout nodes but were especially prevalent near b.vs. (Granules were brown-orange in Sudan IV sections.)
1½ hours:	-	As above.	As above.
3 hours:	Free, extra-cellular, sud.material in some subcap-sular & inter-follicular sinuses of cortex, and in outer medullary sinuses.	Many large oval macrophages with fine sud. granules flooded the inner medulla.	As above.
4½ hours:	Much more free sud.material, penetrating to inner medulla without phago-cytosis than was seen in mesenteric lymph node.	As above. (Maximal in number - inner medulla- at 4½ hours.) (Plate 16,Figure 28)	As above.
6 hours:	Traces remain.	Still many cells, but fewer than at 4½ or 3 hours.	As above.

FERRET : TABLE 19.

Young Ferret : Lymph node from wall of Intestine(Portion F): (3-4 cms. from colon's termination).

	Sudan-Stained Lymph	Reticulo-Endothelial Cells	Migratory Granulocytes
Fasting:	-	Only small groups macrophages with sud. inclusions - chiefly in medullary sinuses. (Droplets of unequal sizes - mask cell details).	Only occasional scattered cells - chiefly in sinus tissue near b.vs.
1½ hours:	- Picture virtually unchanged from that seen in fasting animal.		
3 hours:	-	Phagocytic cells increased in number at 3 hours.	Scattered cells (as above).
4½ hours:	One pale orange coagulum (with Sudan IV) in one part of subcapsular sinus.	As at 3 hours.	As above.
6 hours:	-	?	-

FERRET.

SUMMARY AND DISCUSSION OF RESULTS.

FERRET.

Summary and Discussion of Results:

1. The macroscopic features of the gastro-intestinal tract, together with the dietary habits of the ferret, are described and discussed.

The main features of note are the low-residue contents of the lower bowl, the very short length of colon, and the complete absence of any caecum (Ferret. Plate 1).

2. The chief microscopic feature of note is the presence of villi to within a few centimetres of the gut termination (Ferret. Plate 10, Figure 16).

3. Sites of Absorption. Examination of the results listed, shows that absorption of fat or its split products, apparently occurs, in the younger ferrets, through the mucus-secreting, columnar epithelium of the fundus, body and pyloric region of stomach (Ferret. Plate 4, Figures 5 & 6), from the first part of the duodenum above the entrance of the bile duct (Ferret. Plate 5, Figure 7), and from the terminal non-villous portion of the colon (Ferret. Plate 12, Figures 19-21), as well as from the customary/

customary sites in the small intestine below the entrance of the bile duct.

No such fat absorption picture is seen, with the Sudan dyes, in the stomach, first part of duodenum or colon of the adult animals (Ferret. Plate 11, Figures 17 & 18).

The details and probable significance of these findings are discussed in a later chapter.

4. Migratory Cells. These are heterophil polymorphonuclear leucocytes and the sudanophilic elements are the integral granules (Ferret. Plate 3, Figure 3). They appear to migrate chiefly from submucosal blood vessels and to pass through the stroma of the mucosa towards and eventually through the surface epithelium.

In general these show a tendency, at any one time, to be maximal in the wall of mid-intestine and to diminish gradually as sections pass from this point towards the stomach or towards the terminal colon.

While it is difficult to establish a definite association between numbers, degree of mucosal invasion, and the particular absorptive phase, in any/

any given part of the tract, the general impression gained is that some such relationship may exist, and that the point of maximal involvement is probably around 4½ hours after the ingestion of food. Few, however, are seen at any time in the terminal portions of the intestine.

With regard to their detailed distribution, a glance at the table of results for the adult ferret shows the general trend. Occasional in number in the wall of the stomach, these cells gradually increase in number and degree of mucosal invasion to reach a maximum in the wall of approximately the mid-intestine (Ferret. Plate 8, Figures 12 & 13). They subsequently dwindle till only a few are seen in the wall of the terminal colon(Ferret. Plate 11, Figures 17 & 18).

This trend is more or less traced in the younger series. Throughout the gastro-intestinal tract of the fasting animal, few or no such cells are seen in stomach or duodenum. They make their appearance in the upper part of the tract and reach a maximum in mid-intestine. Few or none are seen in the wall of the two terminal portions.

While/

While increased numbers and distribution are noted at 1½ hours, the gradient is maintained - few being seen in the stomach wall, and these gradually increasing from the upper part of the tract to a maximum in sections from the mid-intestine, and dwindling again to small numbers in portions E & F.

A general and considerable increase in numbers is seen in all upper regions at 3 hours, but again they are maximal in the mid-or lower part of the villøus - containing part of the intestine. They fall sharply from great numbers in mid-intestine to only a very few in the wall of the terminal portions.

Numbers and degree of invasion are, if anything, maximal in all areas at 4½ hours, but again the gradient is maintained and very few occur in the last two portions.

Six hours sees a general diminution in numbers throughout the upper parts of the tract, but numbers are maintained in mid-intestine and dwindle gradually as in the fasting and 1½ hour animals to a very few in the wall of the terminal colon.

While it is difficult to establish a distinct relationship/

relationship between their numbers or degree of mucosal invasion and the post-ingestive phase in the younger animals, it will be seen that any given region tends to show a marked variation.

In the stomach, numbers rose gradually from a few in the base of the mucosa at 1½ hours, to maximal numbers, some showing migration towards the surface and in the lumen at 4½ hours, and a general diminution in numbers and an irregular distribution at 6 hours.

This trend is also seen in the first part of the duodenum though the maximal point is reached perhaps a little earlier (at 3 hours). The third part of duodenum is unusual in that no marked migration or change in numbers is seen till 6 hours.

A fairly uniform number is noted at each post-ingestive phase in portion A and portion C of the intestine. This number, which is increased in portion B, is again uniform throughout the series. Portion D shows some increase at 3 - 4½ hours.

Migratory cells are uniformly scarce throughout the series in portions E and F.

The/

The general impression gained is that, while some results might lead one to suspect a relationship between the numbers of these cells, the degree of their invasion of the mucosa, and the absorptive phase concerned, much more work is required before this can be confirmed or refuted.

5. Stroma and Lymph Vessels.

Active absorption of fat and its passage from epithelium, in the free state, to the stroma and lymph vessels is evidenced in the villus-containing part of the tract, in mature and immature animals.

In addition, lymph vessels show the fat stain in at least one section from the first part of the duodenum at 1½ hours, and in 1½ and 3 hour sections of the terminal colon from the young animals.

Few, if any, macrophage cells of the reticulo-endothelial type are recognised in any sections of the ferret intestinal wall. None are recorded containing Sudan-tinted material.

6. Regional Lymph Nodes.

The large regional lymph node from the root of the mesentery, together with small local nodes in close proximity to the wall of the terminal portions of/

of Intestine (E & F) are examined and described.

In addition photomicrographs are shown of sections from the pyloro-duodenal lymph node from the young ferret (Ferret. Plate 15, Figures 25 & 26).

Three aspects of the picture as seen in Sudan-stained sections are described.

i) The presence or absence of Sudan-stained lymph is recorded.

This is maximal in amount in the mesenteric lymph node after 3 hours and is predominantly found in the sinuses at the cortico-medullary junction.(Ferret. Plate 13, Figures 22 & 23).

ii) Free Reticulo-endothelial macrophages containing sudanophilic inclusions increase in number from a few in the fasting animal to great numbers flooding the sinus tissue especially of the inner medulla at 3 and 4½ hours. (Ferret. Plate 13, Figures 22 & 23). While the inclusion size and the degree of involvement of individual cells are variable in the earlier stages, maximal loading of the greatest number of cells is seen at 4½ hours (Ferret. Plate 14, Figure 24), and the droplets are subsequently (at 6 hours) finer and

of/

of more uniform size.

It appears significant that in these 4½ hour mesenteric lymph nodes, while considerable amounts of free material are seen in sinuses of the cortex and outer medulla, almost all sudanophilic material is apparently phagocytosed by the time it reaches the inner medulla.

iii) Migratory granulocytes similar to those so described in the gut wall, are irregularly scattered throughout the tissues of the node.

As in the intestine, they seem to predominate near the walls of small blood vessels. Their granules, the sudanophilic element, show a characteristically different staining reaction from that seen in the macrophage cells.

A somewhat similar picture presents in the lymph node from the wall of Portion B (Ferret.Plate 16, Figures 27 & 28), but it is significant that much more free material penetrates to the inner medulla at 4½ hours, and presumably leaves the node, without preliminary phagocytosis, than is noted at any period in the mesenteric lymph node.

While/

While there is little if any trace of free, Sudan-stained material in the sinuses of the node from the terminal colon, phagocytic cells with sudanophilic globular inclusions do show a marked numerical increase, in the younger ferrets, at 3 and 4½ hours. This would suggest that some fat has been absorbed by the lymphatic pathway at this site, though the possibility of the mobilisation of depot fat cannot be ruled out.

---

2. MOUSE.

MOUSE.

CONTENTS.

MOUSE.

MATERIALS AND METHODS.

MOUSE.

The mouse was chosen as an example of the herbivorous animal which has now more or less adapted itself to omnivorous dietary habits.

Materials and Methods.

Animals: Ten young adult male mice were selected from the department's colony. Their weights ranged from 25 to 30 grams. Only animals which appeared normally active and showed no signs of pre- or post-mortem pathological changes were used for histological study.

Diet & Experimentation: Each animal was segregated for one week's observation before experimentation. During this time it was given free access to water and was fed on its customary diet - a mixture of Diet 41 (Bruce & Parkes,1949) and Diet 86 (Howie, Rowett Institute, 1950), the composition of which are as follows:

Diet 41:

Wholemeal flour	46 per cent.
Sussex ground oats	40 per cent.
Fish-meal	8 per cent.
Dried yeast	1 per cent.
Dried skimmed milk	3 per cent.
NaCl	1 per cent.
Cod-liver-oil	1 per cent.

Digestible protein	13.6 per cent.
Soluble carbohydrate	48.4 per cent.
Fat	4.5 per cent.
Fibre	1.4 per cent.

Diet 86:

Wheat, whole ground	50 per cent.
Barley, whole ground	25 per cent.
White fish meal	7 per cent.
Meat & bone meal	6 per cent.
Dried brewer's yeast	5 per cent.
Dried grass meal	5 per cent.
Cod liver oil	1 per cent.
Salt	1 per cent.

Moisture	14.3 per cent.
Soluble carbohydrate	53.4 per cent.
Protein	20 per cent.
Fat	3.8 per cent.
Fibre	3.3 per cent.
Ash (Ca 0.7 ; P 0.8)	5.2 per cent.

Two animals were killed without further preparation. The others were fasted overnight - all food, but not water, being removed from the cages for approximately 16 hours - and two were killed in this fasted state. The remaining animals were fed from a pipette and took 1 to 2 c.c. 'skim-cream' each. Thereafter one was killed at each of the stated times - 1½, 3, 4½, 6, and 12 hours. An additional animal was killed at 4½ hours.

As with all other series, where material was frankly unsatisfactory, or results equivocal, further animals/

animals were selected and the experiments repeated.

Each mouse was weighed before and after fasting and again before death. Almost all showed a fall in weight of from 1 to 5 grams on the morning after fasting. For several the loss was maintained or increased by the time of killing. Few regained any of the lost weight in the interval between feeding with 'skim-cream' and the time of death.

Method of Fixation: The abdomen was opened immediately after death by coal-gas, and the tissues removed with the minimum amount of direct handling to 12% neutral formalin, for a period of 24 to 48 hours.

Tissues Removed for Histological Examination:

Portions were taken from the upper and lower parts of the stomach.

The first centimetre of the duodenum (immediately distal to the pylorus) was removed, along with portions from upper and lower jejunum, & the terminal centimetre of the ileum. A portion of the wall of caecum from its thin ballooned out area opposing the ileo-caecal junction; the proximal colon at the hepatic/

hepatic flexure (3 to 4 cms. from the caecum); and the distal colon (2 to 4 cms. from the colonic termination) were also taken for examination.

The relatively large lymph node in the root of the mesentery, which was a constant feature, was secured, and, where found, the minute lymph nodes which occasionally guarded the ileo-caecal entrance.

Sections: These were again cut, at a thickness of from 7 - 10 μ , on the freezing microtome.

Methods of Staining: Sections were stained with Sudan IV and Sudan Black, by the procedures outlined for the ferret. They were mounted in Kaiserling's glycerine jelly and ringed with microscopic cement.

MOUSE.

RESULTS.

MOUSE.

Results:

Macroscopic Features:

The macroscopic features of the mouse gastro-intestinal tract are too well-known to require further description here. For the purposes of this study, it is sufficient to draw attention to the formation of the stomach, which is clearly divisible into an upper portion, which is thin-walled, white, and translucent, and a lower portion, which is thick-walled, red, and opaque. In common with the rat, this organ was rarely found to be empty even after overnight fast.

The caecum is greatly developed as in herbivorous animals, and was always found, at post-mortem, to contain semi-fluid, green, faecal matter.

Microscopic Features:

The histological picture as seen in sections stained with the fat-soluble dyes - Sudan IV and Sudan Black - is presented, for the reader's convenience, in tabular form.

Additional, short, structural notes on the region concerned are appended in brackets where necessary.

Unless/

Unless otherwise stated, the inclusion of any feature in the tables carries with it the implication that it stained positively with both Sudan stains.

The 'Epithelium' referred to is the lining epithelium of the appropriate part of the alimentary tract. It will be noted that here, as in the other species examined, there is functional independence of the villi, and that where involved, the epithelium invariably shows maximum involvement and maximal sudanophilic droplet inclusions in the cells at the tips of the villi. These droplets, where present, are always graded downwards in size from the cells at the tips to those at the base of the villi; and in the individual cell from its luminal to its basal aspect, the largest droplets being deep to the striated border, and the finest adjacent to the basement membrane.

It anticipates discussion of the findings to point out again that, as in the case of the ferret, the cells described here as 'Migratory cells' are in fact granular leucocytes. In this connection only those cells which took up the Sudan stains are recorded under this heading. The sudanophilic elements are invariably the granules. Some indicat-

ion is given not only of the distribution but also of the approximate numbers of these cells seen in sections cut at 7 to 10 μ. These figures are intended only as comparative approximations to give some idea of the time of maximal invasion of the mucosa in each area of the gut wall by these cells.

A negative result recorded under 'Stroma' or 'Lymph Vessel' implies that no sudanophilic material, intracellular or extracellular, apart from that seen in the migratory cells, which in all cases are recorded separately, is seen in the stroma, or in the lymph vessels of the mucosa, submucosa, muscularis, and serosa.

The Time headings are self-explanatory. Each refers to the absorptive stage at which the animal was killed.

Abbreviations and Synonyms, etc., are as indicated for the ferret.

MOUSE : TABLE 1.

Adult Mouse : Forestomach : (Upper sac of mouse stomach. Lined with Stratified Squamous Epithelium - Keratinised).

	Epithelium.	Stroma	Lymph Vessels.	Migratory Cells.
Fasting:	Small, discrete sudanophilic globules in a few localised areas of the Stratum Corneum (Plate 1,Figure 1)	-	-	Sudanophilic granulocytes occasionally present in lamina propria, along inner aspect of basement membrane.
1½ hours:	The Sudan stains diffuse throughout all cornified layers. Free fat adheres to surface. (Plate 2,Figures 3 & 4)	-	-	Very few in number, but similarly distributed.
3 hours:	Staining in Corneum diminishing in amount & density of reaction. (Plate 3,Figure 6)	-	-	Increased numbers similarly situated.
4½ hours:	As at 3 hours. (Plate 3,Figure 7)	-	-	As at 3 hours.
6 hours:	Stain concentrated in surface layers of the corneum.(Small globules in upper layers. ? faulty technique). (Plate 2,Figure 5) Pale,diffuse Sudan colouring throughout corneum.	-	-	Very few present.
12 hours:	Pale, diffuse staining still present with Sudan stains. (Plate 1,Figure 2)	-	-	Very few present.

N.B. Stratum Lucidum is tinted with Sudan-stains and is prominent in all sections due to its high refractility.

MOUSE : TABLE 2.

Adult Mouse : Stomach - Body: (Typical gastric pits, peptic & oxyntic cells).

	Epithelium	Stroma	Lymph Vessels.	Migratory Cells.
Fasting:	-	-	-	-
1½ hours:	No satisfactory material obtained.			
3 hours:	Fine, dust-like infranuclear, sudanophilic granules in surface cells. (Mucus-secreting columnar cells). (Plate 4,Figure 8)	-	-	Scattered cells in lower third of lamina propria between & deep to the base of the test-tube glands. (up to 12 around base 1 gland). A few in submucosa.
4½ hours:	-	-	-	Several in submucosa.
6 hours:	-	-	-	-
12 hours:	-	-	-	-

MOUSE : TABLE 3.

Adult Mouse : Duodenum - First Part: (Above entrance bile duct. Immediately distal to pylorus.)

	Epithelium.	Stroma	Lymph Vessels.	Migratory Cells.
Fasting:	A very few cells at tips villi show fine, sudanophilic granules on basal aspect of mucleus.	-	-	Occasional cells in lamina propria between crypts in lower third of mucosa.(1-3 between any two crypts; some areas negative.)
1½ hours:	No satisfactory material obtained.			
3 hours:	Minute, sudanophilic globules, chiefly infranuclear, in cells at tips of villi. (Plate 5,Figures 9 & 10)	-	-	Occasional cells between crypts, and in stroma at root of villus. A few between acini of Brunner's glands in submucosa. (Plate 6,Figure 11)
4½ hours:	No satisfactory material obtained.			
6 hours:	Almost completely negative. A few cells resemble those seen in fasting animal. (Plate 6,Figure 12)	-	Several submuc. l.vs. stain palely with Sudan. (Plate 6, Figure 12)	several in lamina propria at base of mucosa; a few at tips villi.
12 hours:	Almost entirely negative	-	-	Occasional cells between crypts & in submucosa.

MOUSE : TABLE 4.

Adult Mouse : Jejunum - Upper Third:

	Epithelium	Stroma	Lymph Vessels.	Migratory Cells.
Fasting:	In complete cross section, 1-2 groups of 5-6 cells show 2-3 small drops sudanophilic material in infranuclear position.	A few scattered dust-like particles sudanophilic material.	-	Occasional cells between crypts at base of mucosa.
1½ hours:	Entire epith. filled with sudanophilic globules; largest deep to striated border of the cell; very fine in basal area. (Plate 7, Figures 13 & 14).	Fine, free granules, many near basement membrane of epith. & of same size as those deep to nucleus.	Central lacteal; a few l.vs.+ at point where lacteal joins submuc. network.	Scattered in lower third of mucosa, in the lamina propria. (Plate 7, Figure 13).
3 hours:	Epith. even more densely packed with sudanophilic droplets. Greater variation in size noted. Very large drops deep to striated border, at tips villi. Size progressively diminishes from tip to root of villus. (Plate 8, Figure 15)	As above.	Mucosal-submucosal l.vs. stain with Sudan.	Marked increase in numbers in stroma of villi, and between crypts in lower part of the mucosa. (From 12-20 per villus; most dense in nos. towards its base.) (Plate 10, Figure 21)

MOUSE : TABLE 5.

Adult Mouse : Jejunum - Upper Third: (continued).

	Epithelium	Stroma.	Lymph Vessels.	Migratory Cells
4½ hours:	All cells 'loaded' with sudanophilic globules; grading in size from largest at tips villi to smallest at root, & from large deep to striated border to fine in basal region of each cell. (Plate 8, Figures 16 & 17)	Minute, free, sudan-stained particles in stroma.	L.vs. + between crypts & in submuc-osa.	A few in stroma at tips villi. A few near b.vs. in submucosa. Many in luminal debris.
6 hours:	Much less staining with Sudan. Many villi still show fairly large globules in cells near tip. These are often immediate-ly above nucleus. (Plate 9, Figures 18-20)	Traces only.	Central lacteals & submuc.	Up to 6 between adjacent crypts.
12 hours:	Residual glo-bules in a few cells. (chiefly supra-nuclear). Smaller than nucleus. (Plate 10, Figure 22).		A few lacteals & submuc. l.vs. show traces	2-3 in lamina propria between crypts in lower third mucosa.

MOUSE : TABLE 6.

Adult Mouse : Jejunum - Lower Third: (Villi shorter & mucosa thinner than in upper jejunum).

	Epithelium.	Stroma	Lymph Vessels.	Migratory Cells.
Fasting:	-	-	Only 1 central lacteal+	Several scattered in lamina propria, one or two near tip villus.
1½ hours:	Minute, supranuclear, sudanophilic droplets. Fine, infranuclear 'Dust'. (Plate 11, Figure 23).	Small, free droplets in stroma of villus.	Central lacteals+ Submuc.+ especially at junction with lacteal.	Isolated cells in some areas between crypts. (Not so numerous as in fasting animal)
3 hours:	Entire lining epithelium involved. Globules largest at tips villi. Fine drops also on basal aspect nucleus.	+ (as above)	L.vs.+ at junction mucosa & submucosa	Occasional members between crypts at base of mucosa.
4½ hours:	-"-	+ (as above)	1 central lacteal+	As above. A few in submuc. Some seen in luminal debris.
6 hours:	Considerable staining still present, but much less than at 3 hours. Droplets are also smaller in size. (Plate 11, Figure 24)	+ (as above)	Considerable staining in submucal l.vs. (Plate 11, Figure 24) Some central lacteals+	Only occasional members at tips villi.
12 hours:	Residual droplets present in small groups of epithelial cells.	+ (Traces only)	Central lacteals & at the junction with submucosal l.vs.	3-4 between adjacent crypts.

MOUSE : TABLE 7.

Adult Mouse : Terminal Ileum: (small villi)

	Epithelium.	Stroma	Lymph Vessels.	Migratory Cells.
Fasting:	-	-	-	Occasional members in lamina propria.
1½ hours:	-	-	-	As above.
3 hours:	-	-	-	As above(Lower third of mucosa only).
4½ hours:	Supranuclear sudanophilic globules in many cells. (Approx.to half size nucleus), Fine globules in basal part of cell.	-	Submuc.+	As above.
6 hours:	Chiefly infranuclear globules in those cells involved.	Fine, free droplets in stroma of tip villus.	Several submuc.+	A few between bases of adjacent crypts.
12 hours:	-	Residual traces.	A few l.vs.+ at junction mucosa & submucosa	Several between crypts.Most numerous near b.vs. & l.vs. at junction mucosa & submucosa.

MOUSE : TABLE 8.

Adult Mouse ; Caecum: (No villi)

	Epithelium	Stroma	Lymph Vessels.	Migratory Cells.
Fasting:	Small groups columnar cells at tips mucosal folds show fine, discrete, sudanophilic, infranuclear granules. (Plate 12, Figure 25)	-	-	Only 1-2 between adjacent crypts in deeper zone of mucosa.
1½ hours:	Only one or two cells show the above granules.	-	-	Slight increase in numbers; in lamina propria at base of crypts & in submucosa. (Plate 12, Figure 26)
3 hours:	Fine droplets still occupy infranuclear position in small groups of surface cells.	-	-	Increase, especially in submucosa & at base mucosa.
4½ hours:	Marked increase in epithelial involvement: granules are of varying size, but none exceed ½ nuclear size. Chiefly infranuclear in position. (Plate 13, Figures 27 & 28).	-	-	Only a few in deeper part of lamina propria, & in submucosa especially near b.vs.
6 hours:	No significant change.	-	-	Many more cells seen in submucosa, & strung out in the lamina propria between crypts. Many have penetrated almost to the luminal surface.
12 hours:	As in 'fasting' animal.	-	-	Isolated cells between crypts in deepest part of lamina propria.

MOUSE : TABLE 9.

Adult Mouse : Proximal Colon: (At Hepatic Flexure).

	Epithelium.	Stroma	Lymph Vessels.	Migratory Cells.
Fasting:	-	-	-	Many in submucosa especially where projected into mucosal fold. Also between crypts in mucosa. (Plate 14,Figure 29). Migrating halfway towards luminal surface.
1½ hours:	-	-	-	A few mainly near b.vs. in submucosa especially in core of mucosal fold.
3 hours:	Faint,sudanophilic granularity in a very few lining cells, especially those projecting farthest into lumen of gut.	-	-	A few in lamina propria between crypts; & in submucosal core of mucosal folds.
4½ hours:	Fine,minute & discrete,sudanophilic granules in some surface cells. Chiefly infranuclear in position. (Plate 14,Figure 30)	-	-	-
6 hours:	-	-	-	-
12 hours:	-	-	-	A very few in innermost zone of lamina propria.

MOUSE : TABLE 10.

Adult Mouse : Distal Colon: (2-4 cms. from termination.)

	Epithelium.	Stroma	Lymph Vessels.	Migratory Cells.
Fasting:	-	-	-	-
1½ hours:	-	-	-	Several in lamina propria between crypts & in submucosa.
3 hours:	Tinted granularity with Sudan stains. (Chiefly supranuclear).	-	-	Greatly increased in no.. Most numerous towards base mucosa, but extended ¾ way towards luminal surface. (Plate 15, Figure 31).
4½ hours:	As above.	-	-	Isolated groups in submucosa & adjacent lamina propria of mucosa. No great invasion of mucosa.
6 hours:	As above.	-	-	Very few seen. These are confined to base lamina propria.
12 hours:	-	-	-	As at 6 hours.

MOUSE : TABLE 11.

Adult Mouse : Mesenteric Lymph Node:

	Sudan-Stained Lymph	Reticulo-Endothelial Cells.	Migratory Granulocytes.
Fasting:	Traces in some parts of subcapsular, internodular and medullary sinuses.	A few fixed and free R.E. cells show faint sud. granules in sinus tissue. Reticular cells in some germinal centres are tinted.	The granulocytes (much smaller cells than R.E. macrophages) are scattered irregularly throughout node. Especially prominent near blood vessels. N.B. Qualitatively different staining reaction with Sudan IV to that seen in macrophages.
1½ hours:	Strongly staining lymph floods internodular sinuses of the cortex. Considerable amount has penetrated to medullary sinuses.	Typical large oval macrophage cells in sinus tissue, especially of medulla. Sudanophilic inclusions - discrete & globular.	As above.
3 hours:	Free sudan-stained lymph in almost all sinus tissue.	Cells increased in number. Maximal size of enclosed globules, though wide variation seen.	As above.

MOUSE : TABLE 12.

Adult Mouse : Mesenteric Lymph Node:

	Sudan-Stained Lymph.	Reticulo-Endothelial Cells.	Migratory Granulocytes.
4½ hours:	As at 3 hours.	Cells increasing in number and involvement.	As at 3 hours.
6 hours:	Much less free sudan-stained lymph in all sinuses.	Still great numbers of macrophages in medulla. Sudanophilic droplet inclusions now much finer.	As above.
12 hours:	Little change in picture from that seen at 6 hours.		

N.B. In these nodes the structure is not so regular in arrangement as that found in the ferret nodes, and it is more difficult to draw a clear distinction between cortex and medulla.

Maximal sudan-staining of lymph occurs at this cortico-medullary junction and in the outer medulla.

MOUSE.

SUMMARY AND DISCUSSION OF RESULTS.

MOUSE.

Summary and Discussion of Results:

1. The chief structural feature of note is the presence of the thin-walled, translucent, upper sac of the stomach, with its lining of stratified squamous epithelium. Coupled with this in importance is the noted tendency for food to remain in the stomach of the mouse - as in the rat - for long periods.

2. Sites of Absorption:

These animals were fully mature adults, yet in addition to the accepted sites of fat absorption, sudanophilic material is found in the mucus-secreting columnar epithelium lining the body of the stomach at 3 hours (Mouse. Plate 4, Figure 8), in surface cells of the first part of duodenum above the entrance of the bile duct (Mouse. Plate 5, Figures 9-10), and to quite a marked extent in the lining of the caecal folds (Mouse, Plate 12, Figure 25; Plate 13, Figures 27-28). Even sections from the proximal colon show granules at 3 and 4½ hours (Mouse. Plate 14, Figure 30).

The substance involved in these sites is obviously not neutral fat since none is traced in lymph vessels of the mucosa and submucosa. Granules are angular/

angular and brownish-orange with Sudan IV. This resembles the picture interpreted by both 'lipolytic' and 'partition' schools as due to the presence of fatty-acids.

In addition to this absorption picture in the body of stomach, small discrete globules of sudanophilic material are seen adhering to the surface layers of the keratinised epithelium of the forestomach. Diffuse, pale Sudan-staining occurs throughout the corneum (Mouse. Plates 1-3, Figures 1-7).

The Stratum Lucidum is frequently conspicuous, being highly refractile and distinctly tinted with both Sudan stains.

The probable significance of these findings is discussed more fully in the General Discussion.

3. Migratory Cells.

These are invariably polymorphonuclear leucocytes of the granular class. Many show the kidney-shaped, horse-shoe or ring-shaped nuclei; some appear oval or round.

The sudanophilic element appears to be the integral granule, though these are not distinguishable in paraffin sections stained with Haemalum and Eosin.

The/

The source of origin and the direction of migration are from submucosal and base of mucosa blood vessels, through the lamina propria, towards and through the surface epithelium.

Few, if any, macrophage cells of the reticulo-endothelial class are seen in the stroma of the gut wall itself. Such as are recognised in the submucosa contain ingested fragments of red blood cells and occasionally a whole effete granulocyte, the granules distinctly stained with Sudan, being the only sudanophilic inclusions in these large phagocytic cells.

In no instance are these cells conspicuous or other than infrequent.

In distribution the migratory granulocytes are less predictable than those seen in the ferret. While clear evidence exists of the direction of their migration, no close relationship can be detected between their occurrence in the tissues of the intestinal wall, and the absorptive phase.

In the stomach and small intestine they appear to be maximal in number at 3 and 4½ hours, and indeed very few are seen in earlier and later sections. In no instance/

instance do they approach the large numbers seen in the mid-intestine of the ferret.

In contra-distinction to this, a greater number occur in the wall of the large bowel. No uniformity, however, is achieved in the animals examined, so that although maximal numbers occur in the 6 hour sections from caecum, the peak is reached in the fasting sections of the proximal colon, and at 3 hours in the sections taken from the terminal part of the distal colon.(Mouse. Plate 14, Figure 29; Plate 15, Figure 31).

With the exception of the proximal colon, very few are seen in the intestinal wall of the fasting and 1½ hour animals, and such as occur are chiefly concentrated at the base of the mucosa or within its inner third.

With the exception of the proximal colon, an increase is fairly general at 3 hours.

On the whole they appear to be dwindling to a very few in all regions except caecum at 4½ and 6 hours.

4. Regional Lymph Nodes:

Free Sudan-stained material in the sinuses of the/

the mesenteric lymph node reaches a peak between 3 and 4½ hours. Thereafter this free extracellular material appears to diminish in amount, though traces still remain in the fasting animal.

Free Reticulo-endothelial cells - large rounded or angular, mononuclear cells - with sudanophilic globules of varying size in their cytoplasm, are present in all sections. They increase in number and involvement as absorption of fat proceeds. As the free extracellular material is diminishing in the sinuses, these cells are increasing. Maximal numbers are seen about 6 hours.

Although there is a wide range in droplet inclusion size, largest droplets are seen at 3 hours. They tend to be finer and of more uniform size in the later sections.

It is noteworthy that, in the mouse, more free material is seen within the inner medullary sinuses than occurred in the ferret, where little appeared to penetrate so far without preliminary phagocytosis.

Migratory granulocytes, similar to those in the gut wall, are scattered irregularly and infrequently throughout the tissues of the nodes. Again they are most/

most frequently seen near the walls of small blood vessels.

Their granules have a highly refractile quality and are a brownish-orange colour with Sudan IV.

Macrophage inclusions, on the other hand, are a deep, 'soft' orange colour with the same dye.

---

3. HAMSTER.

HAMSTER.

CONTENTS.

HAMSTER.

MATERIALS AND METHODS.

HAMSTER.

Materials and Methods.

Animals: Five young adult, male, golden hamsters, of weights ranging from 75 to 115 grams, were selected from the Department's colony.

Diet: As for the mice, these were segregated for one week and kept on their standard diet - a mixture of Diet 41 (Bruce and Parkes, 1949), and Diet 86 (Howie, Rowett Institute, 1950), with supplementary corn and fresh greens. Free access to water was allowed at all times.

Experimentation: On the night before experimentation, food, but not water, was withdrawn from the cages. One animal thus prepared was killed in the fasting state. The others were given, from a pipette where possible, 2-4 c.c. of 'skim-cream'. A stomach tube was employed only when other methods were refused.

The animals were subsequently killed, by coal gas, at the customary times - 1½, 3, 4½, and 6 hours.

As before, each animal was weighed at the onset of fasting and before feeding, and again before death. Each showed an overnight loss of weight which ranged from 5-7 grams. Only slight individual variations were/

were noted between this and the weight at the time of killing.

Method of Fixation. To render the small mesenteric lymph nodes more readily visible to the naked eye, and to minimise handling of unfixed tissues, the abdomen was opened immediately after death by a small incision in the upper right quadrant, and about 1 litre of 12% neutral formalin at approximately 40°C., was perfused through the peritoneal cavity at a pressure not exceeding 50 mm. Hg., drainage being provided by a counter incision in the lower left quadrant.

Tissues Removed for Histological Examination.

The organs were left in situ for about 1 hour, after which portions of the stomach wall (upper and lower parts), duodenum (immediately distal to pylorus), mid-jejunum, terminal ileum, caecum, proximal colon (at hepatic flexure), distal colon (4-5 cms. from colonic termination), and the mesenteric lymph node, were removed to 12% neutral formalin for a further 24-48 hours.

By this procedure the lymph nodes were hardened slightly and rendered an opaque white colour before removal from the abdominal cavity.

The/

The apparent advantages of fixation by aortic perfusion were abandoned, after initial experiments had indicated that extracellular sudanophilic material in the sinus tissue of the lymph nodes appeared to be displaced by this method.

Sections: Sections were cut and stained by the methods already described for the Mouse.

HAMSTER.

RESULTS.

HAMSTER.

Results.

Macroscopic Features:

The hamster gastro-intestinal tract presents the features typical of the other herbivorous animals examined.

The stomach is clearly divisible into an upper, white, translucent, thin-walled sac, and a lower, red, thick, opaque, funnel-shaped portion. In animals fed with 'skim-cream', this organ usually contains a little milky-looking fluid.

The caecum is well-developed and contains semi-fluid, green, faecal matter.

Microscopic Features:

The chief structural feature of note is the presence of stratified squamous epithelium, of the keratinised type, lining the upper, translucent half of the stomach. This, however, unlike that in the mouse and the rat, stains only faintly in its stratum corneum with the sudan dyes.

The histological findings referable to the absorption/

absorption of fat, in sections stained with Sudan IV and Sudan Black, are presented in tabular form and summarised later.

Explanatory notes re sub-headings, terminology, etc., are as indicated for the ferret and mouse.

HAMSTER : TABLE 1.

Adult Hamster : Stomach - Upper Sac. (Thin-walled; lined with Stratified Squamous Epithelium of Keratinised type.)

	Epithelium.	Stroma	Lymph Vessels.	Migratory Cells.
Fasting:	-	-	-	Occasional cell in lamina propria on inner aspect of basement membrane of surface epithelium.
1½ hours:	-	-	-	As above.
3 hours:	-	-	-	-
4½ hours:	-	-	-	Occasional cell in lamina propria on inner aspect of basement membrane of surface epithelium.
6 hours:	-	-	-	As above.

N.B. Only pale Sudan tinting occurred in the Stratum Corneum. This showed no change with the absorptive phase.

HAMSTER : TABLE 2.

Adult Hamster : Stomach - Lower Sac. (Thick-walled; funnel shaped lower half of stomach; typical test-tube glands, peptic and oxyntic cells in inner two-thirds of mucosa. The latter cells are tinted by the Sudan stains.)

	Epithelium.	Stroma.	Lymph Vessels.	Migratory Cells.
Fasting:	-	-	-	Isolated members only - between test-tube glands and at base of mucosa, in or near small blood vessels.
1½ hours:	-	-	-	-
3 hours:	-	-	-	-
4½ hours:	-	-	-	One or two in a complete cross section.
6 hours:	-	-	-	As above.(Chiefly in submucosa.)

HAMSTER : TABLE 3.

Adult Hamster : Duodenum (Immediately distal to pylorus.)

	Epithelium	Stroma	Lymph Vessels	Migratory Cells
Fasting:	-	-	Submucosal vessels ++ at junction with lacteals.	Up to 6 in stroma of villus tip. Clusters of 1-2 dozen in submucosa near blood vessels.
1½ hours:	-	-	-	As above.
3 hours:	-	-	-	-
4½ hours:	(Very pale Sudan-stained granularity in some surface cells.)	-	-	Small numbers in lamina propria - some small clusters near blood vessels.
6 hours:	-	-	Submucosal vessels +	Maximal numbers seen in duodenal sections from hamsters. Clusters of 2-3 dozen each near base mucosa.

HAMSTER ; TABLE 4.

Adult Hamster : Jejunum: (Approximately mid-jejunum).

	Epithelium	Stroma	Lymph Vessels	Migratory Cells
Fasting:	Residual pale Sudan colouring in some epith. cells only.	-	Submuc. & lacteal between crypts.	Groups of 1-2 dozen each at irregular & infrequent intervals in base mucosa, near blood vessels. Up to 6 in stroma, tips of some villi.
1½ hours:	Faintly stained droplets (About x2 nucleus) in all epith.cells.	-	-	-
3 hours:	As above.	-	-	Several in stroma of villi.
4½ hours:	Sudanophilic droplets of varying size (Some 4-5x size of nucleus)in cells especially at tips villi.	?	-	Occasional members in submucosa near blood vessels.
6 hours:	Upper Jejunum Very strongly positive picture. All villi involved Maximal droplet size at tips.	Some free droplets.	Central lacteals +	Several in stroma of tips villi.
	Lower Jejunum Droplets very much finer.(Not all villi involved.)	-	Submuc.+ Central lacteals ++	6-12 between adjacent crypts. Several in stroma of tips of villi.

HAMSTER : TABLE 5.

Adult Hamster : Terminal Ileum (At Ileo-caecal junction. Short, slender villi).

	Epithelium	Stroma	Lymph Vessels	Migratory Cells
Fasting:	Faint, Sudan-tinted granularity.	-	-	Up to 6 in stroma villus tip and between adjacent crypts. (Probably maximal numbers seen in ileum of hamster series.)
1½ hours:	As above.	-	-	Smaller numbers, but similarly distributed.
3 hours:	No satisfactory material obtained.			
4½ hours:	Sudan-tinted granularity.	-	Central lacteals +	As at 1½ hours.
6 hours:	-	-	Traces in central lacteals.	As above.

HAMSTER : TABLE 6.

Adult Hamster : Caecum (Thin-walled; No villi.)

	Epithelium	Stroma	Lymph Vessels	Migratory Cells
Fasting:	Sudan tinted granularity in surface cells.	-	-	Up to 12 between adjacent crypts - chiefly towards base of mucosa. (Many show kidney- and almost ring-shaped nuclei.) (Plate 1, Figure 1)
1½ hours:	As above.	-	-	-
3 hours:	Very pale, Sudan-tinted granularity in surface cells.	-	-	Occasional members.
4½ hours:	As above (Supranuclear zone chiefly.)	-	-	As above.
6 hours:	As above. Tinting is still very pale.	-	-	Maximal numbers seen in this region. Similar to those seen in fasting animal, and in ascending colon at 6 hours. Chiefly at base of mucosa. Some in migration through epithelium.

HAMSTER : TABLE 7.

Adult Hamster : Proximal Colon (At Hepatic flexure; 'ascending' colon: No villi.)

	Epithelium	Stroma	Lymph Vessels	Migratory Cells
Fasting:	Pale but distinct Sudan-tinted granularity in surface cells.	-	-	One or two isolated cells between adjacent crypts. Many crypts have none.
1½ hours:	Very faint Sudan-tinted granularity in luminal aspect of surface cells.	-	-	-
3 hours:	As above.	-	-	-
4½ hours:	Very distinct Sudan-tinted granularity in surface cells - in supranuclear & infranuclear positions.	-	-	Occasional members between several crypts.
6 hours:	As above.	-	-	Maximal numbers in this region. Up to 6 between adjacent crypts, chiefly at base of mucosa. Some in submucosal core of mucosal folds. Some in active migration through epithelium. (Plate 1, Figure 2).

HAMSTER : TABLE 8.

Adult Hamster : Distal Colon (4 cms. from its termination: No villi. Much folded mucosa.)

	Epithelium	Stroma	Lymph Vessels	Migratory Cells
Fasting:	Faint but distinct Sudan-tinted granularity above & below nucleus in surface cells.	-	-	One or two in lamina propria between crypts.
1½ hours:	Very faint tinting as above.	-	-	-
3 hours:	As above.	-	-	-
4½ hours:	As above.	-	-	Occasional members only.
6 hours:	Much less distinctly Sudan-tinted than in above sections.	-	-	Up to 6 between adjacent crypts. Some near basement membrane of epith. Near b.vs. in submucosa.

HAMSTER : TABLE 9.

Adult Hamster : Mesenteric Lymph Node:

	Sudan-stained Lymph	Reticulo-Endothelial Cells.	Migratory Granulocytes.
Fasting:	Traces pale-staining lymph in sinuses at cortico-medullary junction.	Large oval or irregularly shaped macrophages in loose lymphatic tissue of medulla and cortex.	Scattered cells throughout the node. Granules brownish-yellow with Sudan IV.
1½ hours:	Traces only. No gross Sudan staining.	Small groups of 5 or 6 each.	As above.
3 hours:	As above.	No great change from that seen at 1½ hours.	As above.
4½ hours:	Isolated traces in subcapsular sinus.	Great increase in macrophages in all sinus tissue.	As above.
6 hours:	Increased amounts in subcapsular sinus. Much has penetrated to some parts of the medulla.	As above. Including subcapsular sinus.	As above.

N.B. The R.E. macrophages on average, are larger cells than any seen in ferret or mouse.

Many of them are crammed with Sudan-stained droplets of widely varying sizes. (For fuller notes, see Summary and Discussion of Results.)

HAMSTER

SUMMARY AND DISCUSSION OF RESULTS.

HAMSTER

Summary and Discussion of Results.

1. Sites of Absorption:

There is no evidence of absorption of neutral fat from the upper or lower sacs of the stomach, the first part of duodenum, caecum, ascending or terminal colon.

Fat absorption as seen in the small intestine is so slight that one is forced to conclude that either efficiency of fat absorption is low in the hamster or that the amounts of cream fed were too small to stimulate an adequate picture of events. Where the mid-jejunum was chosen for examination traces of absorption can be seen at 1½ and 3 hours. In the 4½ hour sections considerable absorption does take place and this resembles the pattern noted in other species. Maximal droplet size occurs in cells at the tips of the villi at 4½ hours and grading downwards occurs in all sections from tip to root of villus and from luminal to basal aspect of the individual cell. Only in the sections taken from the upper jejunum at 6 hours, however, is a strongly/

strongly Sudan-positive picture seen with all lining cells of all villi involved. Here too in the lower jejunum sudanophilic droplet inclusions are very much finer, and not all villi are involved. This would suggest that absorption of fat is almost complete high up in the relatively long jejunum of this predominantly herbivorous animal.

The pale, Sudan-tinted granularity referred to frequently in the lining cells of the rat and guinea-pig caecum and large bowel, etc., is noted in the epithelial cells of some of the sections from the terminal ileum, and in all sections of caecum, ascending and terminal colon of the hamster. While in almost all cases it is too delicate to photograph, yet this appearance is very distinct and quite different from the typical Sudan-stained fat or fatty acid absorption pictures. Its probable significance is discussed later.

2. Migratory Cells:

The migratory cells, in which the sudanophilic elements are again the granules, which stain a typical brownish-orange with Sudan IV, and a dense black with Sudan Black, appear to be leucocytes of the/

the granular class. Further classification has not been attempted. Some cells have the ring-shaped nucleus, and many are of the kidney or horse-shoe shaped type. The cytoplasmic granules are finer than those noted in ferret and mouse.

The preponderance of these cells in, and often around, blood vessels especially of the submucosa, suggest again their origin in, and migration from, such vessels into the tissues of the mucosa itself. Their occasional appearance in the luminal debris and between cells of the epithelium confirms the direction of this migration. (Hamster.Plate 1, Figures 1 & 2).

While it seems reasonable to suppose that their appearance and numbers are in some way related to the digestive processes, no constant association or pattern can be determined. At no time are they present in the great numbers noted in the ferret or, to a lesser extent, in the mouse. They are probably maximal in number in the 6 hour and then in the fasting hamsters. In these specimens they are chiefly present as clusters of one to two dozen near a submucosal blood vessel, and not scattered throughout the/

the lamina propria. It is perhaps of significance to note that the 'wave' of granulocytes appears to be synchronised throughout the small and large intestines.

They are probably maximal, at any one time, in approximately mid-small intestine, diminishing as sections pass from this site towards stomach or towards terminal colon.

In the hamster, isolated free macrophage cells are seen in the submucosa and base of the mucosa in a very few sections, chiefly from the caecum. These cells are oval or elongated and are about 4 to 6 times the size of the granulocytes.

Although not nearly so numerous as in the guinea-pig, the few cells seen in the caecum of the hamster are perhaps more sharply defined in their phagocytic property than any seen in other species. Occasional cells show a whole, though distorted, granulocyte in their cytoplasm. The densely Sudan-stained granules make a sharp contrast to the other highly refractile unstained inclusions, and the amorphous, sometimes Sudan-tinted, particles in the same cell.

There is nothing to suggest that these cells are/

are in active migration towards the epithelium of the intestinal wall.

It is to be stressed that very few of these cells are seen in any of the dozens of sections examined from the hamster material. The majority of sections show none.

3. Lymph Vessels:

It is again noted that in the pyloric end of duodenum (at 6 hours and in the fasting animal) some submucosal lymph vessels contain Sudan-stained material although no active absorption of fat occurs through the epithelium at this site. This appearance which is seen again and again in the rat and the guinea-pig seems to suggest that the vessels of the submucosal lymphatic network are in direct longitudinal continuity, and that some spread of lymph upwards or downwards is possible in this network, without postulating absorption through the epithelium at the site concerned.

4. Regional Lymph Nodes:

The mesenteric was the only lymph node examined from the hamsters.

No gross amounts of free Sudan-stained lymph are seen in these nodes. Traces ih the sinuses, chiefly at the cortico-medullary junction, of the fasting, 1½ and 3 hour animals are increased in amount, extend from subcapsular to medullary sinuses at 4½ hours, and are maximal at 6 hours.

Large, free rounded or irregularly shaped cells of the reticulo-endothelial class are abundant in the loose lymphatic or sinus tissue of the medulla and cortex of the fasting animal.

These cells on average are larger than any seen in the ferret or mouse material. Many of them are filled with sudanophilic droplets of widely varying sizes - this variation in droplet size covering a wider range than any previously noted (in ferret or mouse.)

A number are noted in the subcapsular sinus. These cells seen in the outer zones of the node are larger than those seen in the medulla, and these macrophages in the medulla do not show nearly so much sudanophilic material in their cytoplasm.

Granulocytes similar to those seen in the intestinal wall are scattered in small groups throughout the node. No significant difference in site, number/

number or distribution could be determined at the various post-ingestive times. The granules stain the typical brownish-yellow tint with Sudan IV.

The hamster was fasted overnight for 16½ hours. It seems likely that this, for an animal of its size and dietary habit, is a period longer than can be regarded as 'physiological'. It may be that the macrophage cells recorded here in the nodes of the fasting animal, are already mobilising depot fat, since they exceed in number those seen at 1½ hours and are present with very little free, Sudan-stained lymph.

4. RAT.

RAT.

CONTENTS.

RAT.

MATERIALS AND METHODS.

RAT.

Materials and Methods.

Animals: 9 adult male albino rats, of approximately the same age and weight, were selected from the Department's breeding colony.

Diet: Until the day of experimentation they were fed on a mixture of Diets 41 and 86, and were allowed free access to water.

Experimentation: The experimental procedure was essentially the same as that adopted for the hamsters.

'Skim-cream' was again used, and since the rats invariably refused to take this voluntarily, 6 to 10 c.c. were introduced by stomach tube to each previously fasted animal on the morning of experiment.

Thereafter, one animal was killed at each of the usual post-ingestive times.

Additional animals were sacrificed - two from the colony without any form of preparation, one 3½ hours after 'skim-cream', and a third after a 22 hour fast. This last rat was subjected to a stomach wash-out with warm water, immediately before death.

There/

There was always a slight loss in weight, of from 4 to 5 grams, after fasting. Some of this was usually regained before death.

Tissues Removed for Histological Examination: Portions were taken from regions equivalent to those outlined for the hamster. Additional lymph nodes were secured from the ileo-caecal region.

Methods of Fixation: Preliminary abdominal perfusion was carried out with 12% neutral formalin, by the technique already outlined.

Methods of Staining: Sections were cut on the freezing microtome, and stained with Sudan IV, using Haemalum as a counterstain.

Material from the 22-hour fasted animal, and those killed at 1½ and 3½ hours, was stained by Sudan Black.

Material from the 3½ hour rat was paraffin embedded and sections from these blocks were stained with Haemalum and Eosin.

Frozen sections from several of the specimens were stained by the P.A.S. methods as follows:-

P.A.S./

P.A.S.

1. Tissues fixed in 12% neutral formalin - 24 hours.
2. Frozen sections cut.
3. These were washed in distilled water - several changes.
4. Aqueous Periodic Acid at room temperature - 5 minutes.
5. Rinsed in distilled water - 2 minutes.
6. Rinsed in distilled water - 2 minutes.
7. Schiff's Reagent - 10 minutes.
8. Rinsed in distilled water - 2 minutes.
9. Rinsed in distilled water - 2 minutes.
10. Tap water - 1 minute.
11. Delafield's Haematoxylin - 1½ minutes.
12. Washed and 'blued' in water - 1 minute.
13. Mounted in Kaiserling's Glycerine Jelly or Farrant's Medium.

In an endeavour to minimise tearing gelatine embedding was carried out on the tissues from the 3 and 6 hour animals. After 1 to 2 days in neutral formalin they were incorporated in 10% gelatine at 37 C. (24 hours), and then 20% gelatine for a similar period. After embedding, sections were cut at 5 to 7 μ on the freezing microtome and stained by Sudan IV. This method which seemed to displace extracellular sudanophilic material from the sections, was subsequently abandoned.

RAT.

RESULTS.

RAT.

Results.

Macroscopic Features:

These are too well-known to require enumeration. (See Flower, 1872).

Only two points require accentuation here. The rat stomach, like that of the mouse and hamster, although not distinctly divided into compartments, is marked off into two distinct parts of nearly equal extent by the structure of its walls. The upper has translucent, pale, whitish walls, on naked-eye examination, while the lower, more opaque and muscular wall, shows clearly a reddish-grey vascular and velvety mucosa.

One large ribbon-like lymph node is a constant feature in the root of the mesentery. In the rat this node lies closely applied and parallel to the proximal, ascending colon, and while the mesentery of the small bowel converges on it on one side, a small mesentery links it on the other with this piece of colon.

Two or three, and occasionally up to 4 or 5, small nodes are found guarding the ileo-caecal junction.

In/

In all cases where 'skim-cream' had been given the stomach contained thick curds at post-mortem.

At 1½, 3, 4½ and 6 hours, the mid-jejunum on opening was seen to exude a milky fluid. The lower jejunum at 1½ and 4½ hours was seen to contain bright yellow 'curdled' material. The terminal ileum and caecum in all animals, including the one killed after overnight fasting, contained greenish, faecal matter. The colon was frequently found to be empty.

Microscopic Features:

As in the hamster and the mouse, the upper part of the rat stomach is lined by stratified squamous epithelium of the keratinised type.

The histological findings in this and other areas of the gastro-intestinal tract as seen in Sudan-stained sections are recorded again in tabular form. Unless otherwise stated the inclusion of any feature in the table implies that it stained positively with these dyes.

Some material was stained by the Periodic-Acid-Schiff method. Where this yielded results likely to aid/

aid in interpreting the Sudan-stained picture, these are inserted as footnotes to the appropriate table.

Headings, abbreviations and synonyms are as before.

The stomach washout was carried out in the 22-hour-fasted animal in an endeavour to clear the stomach as far as possible of extraneous fatty materials which might otherwise be adhering to its surface.

In spite of this, the cornified layers of the stratified squamous epithelium lining the upper sac are strongly Sudan-positive.

RAT : TABLE 1.

Adult Rat : Stomach. (Thin-walled upper sac; lined with Stratified squamous epithelium - keratinised.)

	Epith.	Stroma.	Lymph Vessels.	Migratory Cells.
22 hour Fast:	Much of surface epith. shows strongly Sud.pos.material in & adhering to cornified layers. Localised areas - negative.	-	-	Scattered along inner aspect of basement membrane- i.e. in lamina propria. Some in submucosa in & near b.vs. A few between muscle coats - near walls of small b.vs.
"Physiol".Fast:	- Rat. (Plate 1,Figure 2)	-	-	A few in lamina propria deep to basement membrane of surface epith.
3 hours:	Stratum Corneum very strongly positive with Sudan stains. (Plate 1,Figure 1)	-	-	In lamina propria, & submucosa in & near b.vs. (Plate 1,Figure 1)
4½ hours:	Pale diffuse Sudan-staining in Stratum Corneum	-	-	As above. "Strung out" in lamina propria deep to basement membrane.
6 hours:	Pale diffuse (but distinct) Sudan-staining in Stratum Corneum	-	-	-

N.B. 1.) No 1½ hour sections taken from this area.

2.) Sections from the 22-hour-fast, stained with P.A.S. showed a medium-positive reaction in those areas of the Stratum Corneum which were orange in equivalent Sudan IV sections.

RAT : TABLE 2.

Adult Rat : Stomach. (Pyloric end. Thick mucosa with typical test-tube glands, oxyntic cells, etc.)

	Epith.	Stroma	Lymph Vessels	Migratory Cells.
22 hour Fast:	-	-	-	Large numbers at base of mucosa, especially round base test-tube glands. A few in submucosa. No general invasion of mucosa, but some in lamina propria between test-tube glands.
"Physiol".Fast:	-	-	-	Some in lamina propria at base of mucosa & round base of test-tube glands.
1½ hours:	-	-	-	Great numbers in lamina propria, at base mucosa. Some in luminal debris. Only a few seen invading mucosa between test-tube glands.
3 hours:	-	-	-	Great numbers similarly situated. Some in submucosa near b.vs.
4½ hours:	-	-	-	As above.
6 hours:	-	-	-	Still considerable numbers present, but fewer than at 4½ and 3 hours.

N.B. Oxyntic cells show tinted granularity with the Sudan stains.

RAT : TABLE 3.

Adult Rat : Duodenum (Immediately distal to pylorus. Villi present.)

	Epithelium	Stroma	Lymph Vessels	Migratory Cells.
Fasting: ('Physiol'.)	-	-	-	-
1½ hours:	-	-	-	Stroma flooded with cells from base mucosa to tips villi. (100-150 per villus & underlying mucosa.)
3 hours:	? Sudan-tinted granularity. (very pale reaction)	-	-	-?-
4½ hours:	Fine droplets (Sudan-positive) in cells at tips villi.	-	-	Scattered in stroma of villi & mucosa.
6 hours:	Fine droplets in cells at tips of villi. Sudan-stained granularity in other epith. cells (i.e. at sides of villi).	-	Submuc.+	-

RAT : TABLE 4.

Adult Rat : Jejunum. (Approximately mid-jejunum.)

	Epith.	Stroma	Lymph Vessels	Migratory Cells
22 hour Fast:	-	-	-	Stroma of mucosa & submucosa crowded with cells.
Physiol. Fast:	-	-	-	-
1½ hours:	Almost all cells show fine, faintly Sudan-positive 'dust'.	Free droplets in stroma.	Submucos. at junction with central lacteal.	Stroma from base of mucosa to tip of villus flooded with cells. (Up to 200 per villus). (Plate 2, Figure 3)
3 hours:	Minute, discrete, supranuclear, sudanophilic droplets in many cells- chiefly near villus tip. (Average - 6 droplets in 1 cell.) Occasional, infranuclear, sudanophilic granulation. (N.B. Not grossly positive picture; not all villi involved.)	Free droplets in stroma	Central lacteal's continuity traced between crypts to submucosa.	12-20 in lamina propria of each villus. (Many near tips.)

RAT : TABLE 5.

Adult Rat ; Jejunum (continued from previous page).

	Epithelium	Stroma	Lymph Vessels.	Migratory Cells.
4½ hours:	Epithelium covering almost all villi shows minute, discrete droplets - chiefly supranuclear. (About 6 per cell.)	Free droplets in stroma	Submuc. at junction with Central Lacteal.	Up to 25 in stroma of 1 villus and between adjacent crypts.
6 hours:	Almost all villi involved. Largest droplet inclusions at tips villi; smaller at roots of villi. (N.B. Few drops are larger than the nucleus.)	Fewer droplets seen.	Submuc.	-

RAT : TABLE 6.

Adult Rat : Terminal Ileum. (At Ileo-caecal junction. Short, stumpy, irregular & infrequently-placed villi.)

	Epithelium	Stroma	Lymph Vessels	Migratory Cells.
22 hour fast:	-	-	-	Great numbers in stroma villi & especially at base of mucosa. Some in submucosa.
'Physiol.' Fast:	-	-	-	-
1½ hours:	-	-	-	Great numbers in stroma from base mucosa to tips villi. (100-150 per villus).
3 hours:	Some Sudan-positive, supranuc. granulation in cells at tips of villi.	-	-	Still considerable numbers similarly situated - chiefly at base mucosa, but some migrating into stroma of villi, even to tips.
4½ hours:	-	-	-	As above. (Chiefly at base of mucosa. 12-20 around base of each crypt.)
6 hours:	-	-	-	Confined chiefly to inner third of mucosa. A few only in tips villi.

RAT : TABLE 7.

Adult Rat : Caecum. (Thin-walled. No villi.)

	Epithelium	Stroma	Lymph Vessels	Migratory Cells.
22 hour Fast:	-	-	-	Great numbers flood all tissues of the wall.
'Physiol'. Fast:	-	-	-	Several at base of mucosa beside walls of blood vessels.
1½ hours:	-	-	-	Hundreds of these cells flood sub-mucosa & base of mucosa. Some are seen migrating into lamina propria between crypts.
3 hours:	? (Pale, Sud. tinted granularity in surface cells.)	-	-	Maximal numbers seen in this area. (Mucosal & Submucosal stroma flooded)
4½ hours:	As above (Very pale reaction)	-	-	Still great numbers.
6 hours:	-	-	-	As at 4½ hours.

N.B. Haemalum & Eosin sections confirm that the migratory cells are granular leucocytes.

In sections stained with P.A.S. the granules of these cells are P.A.S. positive. A P.A.S. positive granularity is also seen in the luminal aspect of the surface epithelial cells in the 22 hour-fasted animal, and in one kept on ordinary diet and killed without special preparation.

RAT : TABLE 8.

Adult Rat : Proximal Colon. (At hepatic flexure, i.e. 7-8 cms. from caecum. No villi present.)

	Epithelium	Stroma	Lymph Vessels	Migratory Cells.
22 hour Fast:	Faintly Sudan-tinted granularity in surface epithelium	-	-	Up to 12 in lamina propria between adjacent crypts. Many crowded stroma at base of mucosa & in submucosal cores of mucosal folds.
'Physiol'. Fast:	-	-	-	-
1½ hours:	Sudan-tinted granularity in surface epithelium.	-	-	Great numbers in the usual sites - crowd mucosa & submucosa in some areas. (Plate 3, Figure 4).
3 hours:	Sudan-tinted granularity, especially in luminal aspect of cells.	-	- *	Maximal numbers seen in rat tissues. Cells flood lamina propria of mucosa & stroma of submucosa. (Maximal at base of mucosa but extend to surface)
4½ hours:	-	-	-	Much smaller numbers. Chiefly along base of mucosa.
6 hours:	-	-	-	As at 4½ hours. Greatest numbers near b.vs. in base of mucosa.

* Granules are positive in sections stained with P.A.S.

RAT.

Proximal Colon:

N.B. From an additional rat, killed without special preparation, and fixed by the usual method, sections of the proximal colon stained by the P.A.S. method, showed, in addition to strongly stained goblet cells, a positive granularity in the luminal aspect of the surface epithelial cells.

Granulocytes from this animal showed P.A.S. positive granules.

RAT : TABLE 9.

Adult Rat : Distal Colon. (4 cms. from gut termination. No villi. Greatly folded mucosa - each mucosal fold having a core of submucosal tissue. Well-developed muscularis mucosae.)

	Epithelium.	Stroma	Lymph Vessels.	Migratory Cells.
22 hour Fast:	-	-	-	Up to 12 in lamina propria between adjacent crypts. Many crowd the stroma at base of mucosa & in submucosa, especially in core of mucosal folds. Prevalent near submucosal b.vs.
'Physiol'. fast:	-	-	-	-
1½ hours:	-	-	-	As in 22 hour fast, but with few in submucosa.
3 hours:	-	-	-	-
4½ hours:	-	-	-	Up to 6 between adjacent crypts.
6 hours:	-	-	-	As at 4½ hours.

N.B. In rat material, the granular leucocytes, described here as 'migratory cells', commonly show a kidney-shaped, two-lobed, or ring-form nucleus.

Granules are very fine and stain a brownish-yellow colour with Sudan IV.

RAT : TABLE 10.

Adult Rat : Mesenteric Lymph Node:

	Sudan-Stained Lymph	Reticulo-Endothelial Cells.	Migratory Granulocytes.
22 hour Fast:	Traces in a few internodular & medullary sinuses.	Fine, Sudan-stained droplet inclusions in considerable numbers of free R.E. cells in sinus tissue especially of medullary zone. (N.B. No gross staining present -requires careful high power scrutiny to detect).	As many as 70-100 per H.P. field in some areas, chiefly in loose lymphatic (sinus)tissue of medullary zone. (Very dense aggregations of these cells near a fairly large B.V. near hilum, & near smaller vessels on outer aspect of capsule).
Overnight Fast:	A very few palely stained traces in some sinuses.	-	Isolated cells scattered throughout sinus tissue.
1½ hours:	Traces in some parts of the subcapsular sinus, in a few internodular sinuses of the cortical zone, & in the outer portion of the medulla.	Free R.E. macrophages with fine, sudanophilic droplet inclusions, of equal but minute size, crowd the sinus tissue especially of the medullary zone. A few groups of larger, apparently fixed R.E.cells in germinal centres also show stained inclusions.	Great numbers present, but on average fewer per high power field than in 22 hour fast. At their most prevalent towards hilar region.

RAT : TABLE 11.

Mesenteric Lymph Node:

	Sudan-Stained Lymph	Reticulo-Endothelial Cells.	Migratory Granulocytes.
3 hours:	Traces only.	Many free R.E. macrophages with Sudan-stained droplet inclusions flood sinus tissue.	Scattered cells only.
4½ hours:	Subcapsular, many internodular & outer medullary sinuses.	Free R.E.macrophages(Predominantly oval with oval nucleus) showing fine sudanophilic droplet inclusions flood all sinus tissue.	Irregularly distributed as before - chiefly in sinus tissue.
6 hours:	A few traces of palely stained material in 1 or 2 sinuses only.	Only a few, isolated cells show very fine, but discrete, residual droplets of Sudan-stained material. in the loose lymphatic (sinus)tissue especially of medullary zone.	-

N.B. R.E. cell droplet inclusions described are maximal in size at 3-4½ hours and thereafter diminish in size.

In all cases cited, they stain a bright orange colour with Sudan IV.

The granules of the migratory leucocytes on the other hand are a highly refractile, brownish-yellow colour with the same stain.

RAT : TABLE 12.

Adult Rat : Ileo-Caecal Lymph Node:

	Sudan-Stained Lymph	Reticulo-Endothelial Cells.	Migratory Granulocytes.
22 hour Fast:	-	-	These cells flood the node - especially the sinus tissue. (40-50 per high power field.) Maximal concentrations of the cells are seen near the walls of blood vessels.
'Physiol'. Fast:	-	-	-
1½ hours:	Very slight but definite traces in some sinuses of cortex.	Several small groups of R.E.macrophages with sudanophilic droplets in cytoplasm scattered throughout node.	Isolated cells only.
4½ hours:	Traces in some sinuses but not many.	Almost completely negative to Sudan stains.	Isolated members scattered throughout node. (not many).

N.B. No ileo-caecal nodes found in 3 & 6 hour animals.

RAT.

SUMMARY AND DISCUSSION OF RESULTS.

RAT.

Summary and Discussion of Results.

1. Macroscopic and microscopic Features.

The presence of the upper translucent part of the stomach, and its lining of keratinised, stratified squamous epithelium, have already been emphasised.

In all cases where 'skim-cream' had been given the stomach contained thick curds at post-mortem.

2. Sites of Absorption.

The rats did not take kindly to the administration of milk. In no circumstances would they take it voluntarily, and several showed a tendency to regurgitation after forced-feeding.

In spite of the long stay afforded to the curds in the stomach, there is no evidence of absorption of any sudanophilic material through the epithelium lining the lower half of this organ. Only the oxyntic cells show a tinted granularity with the Sudan stains.

In the upper sac the stratum corneum shows a variable response to the dye (Rat. Plate 1, Figures 1 & 2). The P.A.S. reaction in the 22-hour fasted animal is strongly indicative of the substance concerned being/

being neither neutral fat nor fatty acid. This point is discussed more fully in the General Discussion.

Very fine droplets or granules are seen in a few sections from the upper part of the duodenum.

Apart from this, no fat or fatty acid absorption picture is seen in any but the upper part of the jejunum.

Of the areas examined, only the jejunum shows a marked picture of absorption. A faint sudanophilic granulation in the earlier sections taken from this region gives way to minute, discrete droplets, which increase and are maximal in size, and in epithelial involvement, in the later sections.

In most cases absorption of fat, or/& its split products, appears to be complete before the terminal ileum is reached. Only one section of this shows sudanophilic granulation in a few cells at the tips of some villi, and the lymph node from the ileo-caecal region holds only traces of the stain.

There is nothing to suggest absorption of dietary fat or fatty acid in caecum or the large bowel.

The Sudan-tinted granularity recorded in the latter/

latter areas is a general cytoplasmic colouring, only slightly deeper than that general to the section. Its concomitant reaction with P.A.S. would perhaps indicate the high phospholipide content of the cell.

3. Migratory Cells.

These are polymorphonuclear leucocytes of the granular class. Many show the ring-shaped form of nucleus. Granules which are fine and discrete are positive with Sudan and P.A.S. stains.(Rat. Plate 2, Figure 3). In Sudan IV sections their brownish-yellow colour is characteristically different from the typical orange colour of neutral fat. In haemalum and eosin sections the cytoplasm is eosinophilic but no granules can be distinguished.

The cells appear to originate in blood vessels at the base of mucosa and submucosa. Their occasional presence in the epithelium and within the lumen of the bowel suggests the direction of their migration.

More are seen in the rat sections than occur in mouse and hamster material.

Very few, however, are seen throughout the wall of the overnight-fasted intestine. On the other hand, all tissues are flooded with these cells in the animal which/

which was subjected to the 22 hour fast and the subsequent stomach wash-out.

It is significant that the 'wave' of migratory granulocytes appears to involve the whole gastro-intestinal tract at one and the same time. In this animal only (the 22 hour fast), are cells of this class seen invading the smooth muscle coats. This raises the question of the influence of factors such as stress, distension or micro-organisms on the invasion of the intestinal wall by leucocytes.

In almost all sections from the rat, the general impression gained is that migration is 'synchronised' - the whole tract being involved at any one time in a 'wave' of wandering leucocytes.

Maximal numbers are seen in caecum and proximal colon but no clear-cut relationship to the absorptive phase is noted. (Rat. Plate 3, Figure 4).

No large mononuclear macrophage cells are detected in the intestinal wall of these rats.

4. Regional Lymph Nodes.

The mesenteric and ileo-caecal lymph nodes were examined.

Free,/

Free, extracellular, Sudan-stained material in the sinus tissue is maximal at 4½ hours in the mesenteric lymph node.

In the rat this coincides with the greatest number of free reticulo-endothelial macrophages in the medullary zone, and with the greatest degree of their involvement with sudanophilic droplets.

The mesenteric nodes are never entirely negative for free or intracellular Sudan-stained material.

With the exception of the 1½ hour animal, and that fasted for 22 hours, migratory, Sudan-stained granulocytes are irregularly and infrequently scattered throughout the tissues of the node.

In common with the wall of the intestine, the tissues of both mesenteric and ileo-caecal nodes are flooded with these cells in the animal fasted for 22 hours.

Their prevalence near the wall of hilar blood vessels suggests the site of their origin.

The ileo-caecal nodes show traces of Sudan-stained lymph, and only small groups of 'positive' free macrophages occur in their sinuses. This suggests,

as/

as the epithelial absorption picture does, that absorption of fat is complete in these animals high in the intestinal tract, little or none penetrating to the lower ileum for absorption there.

In the 22-hour fasted animal, but not in that subjected to 'physiological' fast, considerable numbers of free macrophages with Sudan-stained droplet inclusions have made their reappearance in the sinus tissue especially of the medullary zone. This agrees with the findings of Poulain (1902) who noted that after prolonged (non-physiological)fasting, macrophages made their reappearance, though little or no free sudanophilic material was to be seen in the sinuses. In this way they appeared to be the instruments in the mobilisation of the depot fat.

5. GUINEA-PIG.

GUINEA-PIG.

CONTENTS.

GUINEA-PIG.

MATERIALS AND METHODS.

GUINEA-PIG.

The guinea-pig was chosen as representative of the true herbivore, as an animal in whose diet, fat normally plays a very small part, and whose efficiency, perhaps even mechanism, of fat absorption may differ markedly from that of the other species examined.

Although presented last, in sequence from the pure carnivore through the omnivores to the pure herbivore, the guinea-pig material was in fact prepared early in the study, and for this reason a slightly different approach was adopted.

Only as the investigation proceeded did it become obvious that it should be extended to include the first part of the duodenum, the upper as well as the lower part of the stomach wall, and the wall of the proximal and distal colon.

In the earlier guinea-pig experiments, therefore, these areas were not examined.

Similarly, it became apparent that Sudan Black widened the scope of the histological study, clarifying often/

often a picture which was otherwise equivocal, because delicate, with Sudan IV.

On the other hand, the Periodic-Acid-Schiff method was used more extensively in this than in later series. Its value, however, only emerged late in the study, and though limited, served to exclude, by its positive reaction in certain areas, neutral fat and fatty acids in material which also stained positively with the traditional Sudan stains.

Some material was fixed by the traditional Osmium Tetroxide method from fasting, 1½, 3, 4½ and 6 hour animals. The obvious difficulty of fixing relatively large pieces of tissue, such as whole lymph nodes, by this method, proved it of limited value.

Each experiment was repeated at least three times.

Materials and Methods:

Animals, Diet and Experimentation:

Eighteen young adult male guinea-pigs were selected from the Department's breeding colony. Their weights ranged from 600 to 900 grams.

These were segregated for one week before the onset of the experiment. During this time they had free access to water and green vegetables, and continued to be fed on the standard animal house diet - Diet 18 (Bruce and Parkes), composition as follows:

Diet 18:

Protein	16.5%
Fat	4.6%
Soluble Carbohydrate	33.7%
Fibre	6.7%

At the end of this period three animals were killed without further preparation. The others were fasted overnight, all food being removed from the cages for a period of 15 to 17½ hours. Free access to water was permitted at all times.

Three animals were killed in the fasting state.

The remaining animals were fed 20 to 30 c.c. 'skim-cream'. Many took this voluntarily from a saucer. The others fed readily from a pipette. Thereafter, three were killed at each of the times - 1½, 3, 4½ and 6 hours.

All animals were weighed before and after fasting, and/

and again before death. Any showing a disproportionate loss in weight were discarded and the experiment repeated with substitutes.

Methods of Fixation, Sectioning, and Staining:

Fixation was by 12% neutral formalin, initially introduced to the abdominal cavity by the method described for the hamster.

Frozen sections were stained with Sudan IV, and others with P.A.S., by the methods already described.

From the material of one animal killed at each of the stated time intervals, after fasting or feeding, sections were stained with Sudan Black, some incorporating the haemalum counterstain.

Tissues Removed for Histological Examination:

From this last series, the study included material from fundus and lower body of stomach, first part of duodenum, immediately distal to the pylorus, upper and lower parts of jejunum, ileum, caecum, proximal (12 cms. from caecum) and distal (5 cms. from colonic termination) colon, and root of mesentery lymph node.

From the two earlier series of male animals killed in the fasting state or after the ingestion of 'skim-cream',/

cream', as well as from all other animals examined, the tissues removed for examination consisted of body of stomach, together with the lymph node guarding the pyloro-duodenal junction, mid-jejunum, and the lymph node in the root of the mesentery, terminal ileum, caecum, with the lymph nodes guarding the ileo-caecal junction.

One animal killed at 4½ hours was fixed by Aortic perfusion of 12% neutral formalin. Frozen sections were stained by both Sudan methods.

Material from one animal killed without any special preparation, was paraffin embedded and sections stained by haemalum and eosin.

GUINEA-PIG.

RESULTS.

GUINEA-PIG.

RESULTS.

Macroscopic Features:

Due to the feeding habits of these animals the stomach, and indeed the whole gastro-intestinal tract, was never found to be empty in animals killed without special preparation.

After overnight fasting, the upper alimentary tract was almost emptied of food or its residues; the stomach was distended with gas, and the caecum and lower colon contained semi-fluid, faecal matter.

At 1½, 3, 4½, and 6 hours, the stomach and upper intestine contained clotted cream. The caecum and colon were still full of fluid, green, faecal matter.

Microscopic Features:

These are presented in tabular form. To facilitate reconstruction of the picture with regard to the distribution of the migratory granulocytes, these are again listed under a separate heading. Other free cells, such as macrophages containing Sudan-stained or other inclusions, are included, where applicable, under the heading 'Stroma'.

A conspicuous feature of the guinea-pig material

is/

is the presence of 'nests' of these large, mononuclear cells apparently of the reticulo-endothelial type, in the lamina propria of the mucosa. These are larger and much more conspicuous than any seen in the wall of the gut in any of the other species examined. Occasional members are visible in the submucosa, but the very prominent groups seen deep to the marginal capillaries of the villi, and between crypts in the non-villous parts of the tract, are constant in position and do not appear in these sections to migrate either towards the epithelium, or indeed in any other direction. Their chief function appears to be the ingestion of effete cells, such as the granulocytes with their sudanophilic granules, and red blood cells. Other amorphous material in their cytoplasm may represent absorbed substances of dietary origin.

Their definite reaction with the Periodic-Acid-Schiff method, together with the very pale tinting of their cytoplasm with the Sudan stains, may be an expression of their pigmented (Lipofucsin)nature.(Pearse, 1953).

One other feature not met with in the other animals is the frequent failure on the part of the granulocytes of the gut wall to stain with Sudan. In sections stained/

stained with Sudan IV they tend often to be highly refractile and dark, or frankly basophilic when counter-stained with Haemalum.

As in the ferret one or two cells in the crypt epithelium in various parts of the intestine are strongly basophilic. Their situation strongly suggests that they are Paneth cells. Although not sudanophilic, these are recorded, where present, in the following tables.

GUINEA-PIG : TABLE 1.

Adult Guinea-pig : Stomach. (Body)

	Epithelium	Stroma	Lymph Vessels	Migratory Cells
Normal: (Ord.diet)	-	-	-	A few isolated groups of cells at base of mucosa.
Fasting:	-	-	-	As above.(Groups of 3 or 4 cells each).
1½ hours:	-	-	-	-
3 hours:	-	-	-	Occasional cells in lamina propria at base of mucosa.
4½ hours:	-	-	-	As above.
6 hours:	-	-	-	-

N.B. The granules of the leucocytes are P.A.S.+ as well as sudanophilic.

GUINEA-PIG : TABLE 2.

Adult Guinea-pig : Duodenum (First Part). (Brunner's Glands visible).

	Epithelium	Stroma	Lymph Vessels	Migratory Granulocytes
Fasting:	Very faint Sud. tinting in upper part of lining cells. (Bright basophilic cells in crypt epithelium (2–4 per crypt). (Plate 1, Figures 1-2) Occasional isolated basophilic cells set in epith. at sides of villi)	Large macro-phage cells in tips some villi. Not Sudan positive. ? effete r.b.cs. ingested.	Submuc.	Up to 6 per villus and associated stroma between crypts. (granules brownish-yellow with Sudan IV).

GUINEA-PIG : TABLE 3.

Adult Guinea-pig : Duodenum (First Part). (Villi infrequent and 'stumpy'; Brunner's Glands commencing).

	Epithelium	Stroma	Lymph Vessels	Migratory Granulocytes.
6 hours:	Fine sudanophilic, supranuclear & infranuclear granules in surface cells. (Basophilic cells in crypt epithelium. (2-3 / crypt))		-	Granulocytes in core villi and in lamina propria between crypts, at base of mucosa and in submucosa. (Up to 20 associated with 2 adjacent crypts and 1 villus). Granules are brownish-orange with Sudan IV.

N.B. Only Fasting and 6 hour specimens secured from this region.

GUINEA-PIG : TABLE 4.

Adult Guinea-pig : Jejunum (Mid). (Approximately mid-jejunum; much-folded mucosa; each fold with submucosal core, and long slender villi arising from and covering this).

	Epithelium	Stroma	Lymph Vessels	Migratory Cells
Fasting:	- (Basophilic cells in crypt epith.)	A few large pale cells in tips villi.	-	From 6-12 cells between crypts, chiefly at base of mucosa.
1½ hours:	Many villi involved, but picture delicate. Fine droplets in cells at tips of some villi. (In a few, some droplets = size of nucleus). Very fine granules on basal aspect nucleus. (Plate 3, Figure 4)	As above. Some cells contain broken down r.b.cs. etc. Some inclusions are darkened by Sudan Black.	Central Lacteals.	Scattered cells in lamina propria.
3 hours:	Very fine droplets in cells at tips of villi. (up to 8 droplets above nucleus in one cell). Fine infra-nuclear sudanophilic 'dust'. Not all villi involved.	Fine sud. 'dust' in stroma. Large cells as above.	Central Lacteals (Plate 3, Figure 5) Submuc. l.vs. are sud. positive.	Up to 6 at base of mucosa between adjacent crypts.

GUINEA-PIG : TABLE 5.

Adult Guinea-pig : Jejunum (Continued).

	Epithelium	Stroma	Lymph Vessels	Migratory Granulocytes
4½ hours:	All cells involved. Maximal epith. involvement and droplet size, in cells at tips villi. (Plate 4, Figure 6) (No very large drops seen - none exceed twice the nuclear size). Fine infranuclear 'dust'. (Basophils in crypt epith) (Plate 2, Figure 3)	Fine, free sud. material.	Central lacteals, Submucosal & intramusc. vessels are sud. positive. (Plate 4, Figure 6)	Up to 20 per villus and associated lamina propria between crypts. (Predominantly at base of mucosa though some migration is taking place).
6 hours:	Considerable number of villi still involved, but picture not so grossly positive with Sudan stains. Maximal droplet size seen in these sections - some equal nucleus; a few are larger.	As above.	As above.	Similar in numbers and site to 4½ hour picture.

GUINEA-PIG : TABLE 6.

Adult Guinea-pig : Jejunum (Continued).

	Epithelium	Stroma	Lymph Vessels	Migratory Granulocytes
Normal Diet:	Fine, discrete sudanophilic 'dust' in supranuclear zone of lining cells most villi. (N.B. No large droplet inclusions seen).	Occasional large pale cells with P.A.S.+ and Sud. inclusions in stroma at tips villi. (Some villi show quite a number of these cells - up to 12)	Occasional central lacteal & submuc. l.v.	1 or 2 at base mucosa.

N.B. Some inclusions in macrophage cells appear to be fragments of red blood cells.

GUINEA-PIG : TABLE 7.

Adult Guinea-pig : Terminal Ileum. (Short, stumpy villi. Much-folded mucosa - each fold with a sub-mucosal core).

	Epithelium	Stroma	Lymph Vessels	Migratory Cells.
Fasting:	With Sudan Black -slightly darkened granulation in luminal aspect of surface cells.	Small groups large oval cells in stroma at tips villi. (?ingested broken down r.b.cs. - negative to Sudan stains)	?submuc.	Up to 6 in lamina propria between adjacent crypts, and about 3 in core of each villus. (N.B. Granules a definite brownish-orange with Sudan IV).
1½ hours:	Sudan Black granulation as above. With Sudan IV fine, supranuc. droplets in some cells only, especially near tips of villi.	Large oval cells with clear oval nucleus, situated as above, show darkened granules with Sudan Black. A few are seen in submucosa.	Submuc.	From 6-12 in lamina propria between adjacent crypts. Very few in stroma of villi. (Plate 5, Figure 7).
3 hours:	Minute, discrete sudanophilic droplets of unequal but very small size are seen in some cells. (About 6-8 droplets in each cell involved, in area between nucleus & striated border. Also fine, infra-nuc. Sudan-stained 'dust' in some cells, especially at tips villi.		Submuc.	Up to 12 between adjacent crypts. A few in core of villi. Many at base mucosa round base of crypts. (N.B. Numbers greater than in earlier sections).

GUINEA-PIG : TABLE 8.

Adult Guinea-pig : Terminal Ileum.

	Epithelium	Stroma	Lymph Vessels	Migratory Cells.
4½ hours:	Maximal epith. involvement. No large droplets seen. All much smaller than nucleus. Largest at tips villi & in supranuclear position. Smaller infranuc.	Fine free dust	Central lacteals.	Maximal numbers seen. Few in villi; most at base mucosa & migrating between adjacent crypts. (up to 20 between 2 crypts).
6 hours:	As at 4½ hours, but several droplets at tips villi are larger than any seen at 3 or 4½ hours. Again infranuclear Sudan-stained granulation.	As above.	Central lacteals: Submuc.	Up to 6 in stroma of each villus & between adjacent crypts. Great numbers in submucosa where this is projected into core of mucosal fold.
Ord. Diet:	? Faint positive granulation in some surface cells in luminal aspect.	Small groups (5 or 6 cells in each) * large cells in stroma at tips villi. Show faintly positive granules with Sud. IV.	-	(Some granulocytes, but granules basophilic not Sudan-positive in these sections).

Footnote: These "large cells" in stroma of villi are 4-6 times larger than the migratory granulocytes.

* N.B. In these large cells the granules which are faintly tinted with Sudan IV are P.A.S.positive with the appropriate stain.

GUINEA-PIG : TABLE 9.

Adult Guinea-pig : Caecum (Thin-walled. No villi).

	Epithelium	Stroma	Lymph Vessels	Migratory Cells
Fasting:	Very faint Sudan-tinted granularity in luminal aspect surface cells.	3-8 large cells with clear, refractile droplet inclusions - some resembling in staining reaction broken down r.b.cs.; others unidentifiable - in stroma between adjacent crypts. (Very distinct with Sudan Black).	-	Occasional granulocytes in lamina propria between crypts, especially at base of mucosa.
1½ hours:	-	2-3 such cells in mid-lamina propria between adjacent crypts. Pale granulation in cytoplasm.	-	1 or 2 at base of lamina propria between adjacent crypts.
3 hours:	Very pale orange granularity (With Sudan IV) in supranuc. zone surface cells.	As at 1½ hours.	1 sub-muc. vessel with pale orange staining (Sud.IV)	-

GUINEA-PIG : TABLE 10.

Adult Guinea-pig : Caecum.

	Epithelium	Stroma	Lymph Vessels	Migratory Cells.
4½ hours:	Sudan-stained granularity in surface cells - in luminal aspect.	Many large oval or angular cells with Sudan positive granules in their cytoplasm in lamina propria between crypts.	-	Many in migration through the epithelium. (These cells show fine, discrete, brownish-orange granules with Sudan IV).
6 hours:	?Fine, faint Sudan-stained granularity luminal aspect of nuclei of surface cells.	Again large pale cells, similar site, numbers, & staining reaction.	-	Some at base of mucosa especially near walls of blood vessels. (2-3 between bases of adjacent crypts).
Ord. Diet:	Faint yellowish granularity in luminal aspect of surface cells with Sud.IV.	Large cells seen in stroma. (Plate 6, Figures 8-10)	-	1 or 2 at base of lamina propria between adjacent crypts.

N.B. 1. The large, pale, oval or angular phagocytic cells with the clear, open nuclei, are invariably present in nests of 7-8, and are found in the stroma of the mucosa deep to the surface epithelium and its underlying marginal capillaries. They are 4-6 times the size of the granulocytes.

The contained granular or globular material is frequently P.A.S. positive, the reaction in such sections being subtly different in its colour quality from that in the goblet cells. Although somewhat similar to that seen in the granular, supranuclear zone of the surface epithelial cells, it tends to be darker, and slightly brighter in these large, apparently free phagocytic cells.

The crypts are widely spaced from each other.

GUINEA-PIG : TABLE 11.

Adult Guinea-pig : Distal Colon (5 cms. from termination).

	Epithelium	Stroma	Lymph Vessels	Migratory Granulocytes.
Fasting:	Sudan-tinted supranuclear granularity in cell cytoplasm,	Occasional large pale macrophage cells deep to marginal capillaries in stroma - apparent sudanophilic granulation yet only lightly tinted, not frankly orange with Sudan IV. Some inclusions resemble r.b.cs. in colour reaction.	-	A few isolated cells at base of mucosa & in lamina propria between crypts.
4 hours:	Very faint Sudan-tinted granularity in some surface cells (luminal aspeet).	As above.		As above. Several in submucosa near & in B.Vs.

GUINEA-PIG : TABLE 12.

Adult Guinea-pig : Pyloro-Duodenal Lymph Node:

	Sudan Stained Lymph	Reticulo-Endothelial Cells.	Migratory Granulocytes.
Fasting:	-	A very few, free macrophages with Sudan-stained 'dust' in their cytoplasm. (1 per High Power field) - especially medullary sinuses.	Up to 20 per High Power field. in loose lymphatic (sinus) tissue. especially in medulla. (Granules are orange-brown in Sudan IV).
1½ hours:	Very pale orange coagulum in a few sinuses with Sudan IV.	Almost completely negative. Only scattered members with brownish inclusion granules in Sudan IV.	(30-40 per High Power field with Sudan Black. All sinus tissue flooded with these cells).
3 hours:	Pale orange coagulum in 1 or 2 sinuses only.	Free macrophages (rounded or angular cells, rounded or oval nucleus) in loose lymphatic tissue especially of medulla, show frank, Sudan-positive globules, of varying but all small sizes. About 6-10 H.P. field in medullary sinuses.	Granulocytes flood loose lymphatic tissue - about 20-50 per High Power field in medulla. Granules are grey-green and refractile in Sudan IV sections.

GUINEA-PIG: TABLE 13.

Adult Guinea-pig : Pyloro-Duodenal Lymph Node:

	Sudan Stained Lymph	Reticulo-Endothelial Cells	Migratory Granulocytes.
4½ hours:	Traces only, chiefly in some internodal sinuses & outer medullary sinuses. (picture not grossly Sud.positive).	Free macrophages still found in medullary sinuses. Sudan-stained 'dust' in their cytoplasm is much finer than globules seen at 3 hours. Although there are still considerable numbers, the cells are not so conspicuous as at 3 hours - because of the droplet size.	Granulocytes very prevalent in loose lymphatic tissue and dense lymphatic tissue - especially numerous in outer medullary zone. (80-90 in some High Power fields in this zone).
6 hours:	Traces in some internodal and outer medullary sinuses.		12-20 per High Power field in outer area of the medulla.

GUINEA-PIG : TABLE 14.

Adult Guinea-pig : Pyloro-Duodenal Lymph Node:

	Sudan Stained Lymph	Reticulo-Endothelial Cells	Migratory Granulocytes
Normal Diet:	(Pale, bright magenta-stained lymph in some internodal & outer medullary sinuses in P.A.S.) (This coagulated lymph seen in equivalent Sudan IV. sections is not sudanophilic). One animal did show Sud.+lymph in these sinuses.	(Great, many free R.E. cells with dense purple granules in P.A.S. These are mainly in sinus tissue especially of medulla. N.B. Varying degree of involvement of individual cells - some are filled to capacity; others show only fine 'dust' in cytoplasm, and are smaller cells) Many R.E. cells in sinus tissue show fine sudanophilic granulation. Droplet inclusions of varying, but all very minute size, are very distinct.	Many granulocytes with highly refractile, darkened, granules, but not Sudanophilic, seen in loose lymphatic (sinus) tissue in Sudan sections. (Many with Bilobed nuclei).

GUINEA-PIG : TABLE 15.

Adult Guinea-pig : Root of Mesentery Lymph Node:

	Sudanophilic Lymph	R.E.Cells	Migratory Granulocytes.
Fasting:	Traces in some parts subcapsular sinus, some internod., and in medullary sinuses (especially outer medulla).	Several with Sudan+ droplets in sinus tissue. Especially prevalent in medulla towards hilum-up to 12/H.P.field. Very pale. Many inclusions resemble broken down r.b.cs. etc. **Not fat.**	Scattered cells with brownish-yellow granules in Sud. IV. (Up to 20 per H.P.field in medulla with Sudan Black).
1½ hours:	As above. (Not grossly positive picture)	Faintly positive fine 'dust' in some free cells, chiefly in medulla. (Delicate picture; requires H.P.scrutiny to detect).	Isolated. Scattered.
3 hours:	Almost all sinus tissue throughout node shows free, Sudan+ material. (Maximal amount)	As above, but many cells involved. Delicate but distinct discrete droplets.	Up to 50 per H.P.field in medulla. Granules refractile and dark in Sudan IV. Maximal numbers seen.
4½ hours:	Still considerable amounts but not so much as at 3 hours. (Not all sinuses involved).	Maximal numbers. Droplet inclusions larger. In loose lymphatic tissue especially of medulla.	Comparable numbers and sites as at 3 hours.
6 hours:	Traces in subcap. and internodular sinuses. Considerable quantity in outer medullary sinuses.	Fine+ 'dust' in some cells. (Least distinct picture seen).	Up to 12 per H.P.field in loose lymphatic tissue. Granules are darkened in Sudan IV. sections.
Normal:	Sinuses in some areas (Esp. outer medulla) show+ lymph. Some parts subcap.: other areas negative.	Many, free macrophages; droplets of varying, but small, sizes.	—

GUINEA-PIG : TABLE 16.

Adult Guinea-pig : Ileo-Caecal Lymph Node:

	Sudanophilic Lymph in Sinuses.	Reticulo-Endothelial Cells.	Granulocytes.
Fasting:	-	Free Macrophages with ingested effete r.b.cs. in sinus tissue.	Isolated cells only - scattered throughout node.
1½ hours:	-	A few groups of large oval, rounded or irregularly shaped macrophages with Sudan-stained globules in sinuses.	Isolated cells - scattered irregularly throughout loose lymphatic tissue. (N.B. Granules not Sudan+, but a dark grey-green in sections stained with Sudan IV).
3 hours:	-	No grossly positive picture. Some free cells with Sudan-stained droplets, chiefly in sinus tissue, but a few in lymphatic nodules.	As above.
4½ hours:	-	Some, especially near hilar region. Not many and not distinctly Sudan-positive. Reaction - brownish yellow.	Loose lymphatic tissue flooded with these cells- about 30 per High Power field in medulla. (Granules dark, not Sudan+)
6 hours:	-	Free macrophages in sinuses contain no definite Sudan-positive material. Some brownish-yellow. Some with ingested broken r.b.cs. in medulla.	A few scattered throughout node, but chiefly in hilar region.
Normal:	-	Occasional free cell with fine Sudan+ 'dust'.	Occasional only.

GUINEA-PIG.

SUMMARY AND DISCUSSION OF RESULTS.

GUINEA-PIG.

Summary and Discussion of Results:

1. Microscopic Feature of note in the guinea-pig intestine is the presence of very prominently basophilic cells in the epithelium at the base of the crypts of Lieberkuhn. (Guinea-pig. Plate 1, Figures 1 & 2 ; Plate 2, Figure 3).

These cells are epithelial cells, and the basophilic element is the cytoplasmic granule. In site and numbers they resemble the Paneth cells.

2. Sites of Absorption.

There is no evidence of absorption of fat, or its split products, as judged in sections stained by Sudan, in any but those from the small intestine.

Only fine sudanophilic granulation is seen in the first part of the duodenum.

Absorption continues throughout the jejunum and ileum and stops abruptly at the commencement of the large bowel.

Within the jejunum itself fine droplets at 1½ hours, increase in size to reach a maximum at 6 hours. Epithelial/

Epithelial involvement, on the other hand, reaches its peak at 4½ hours. (Guinea-pig. Plate 4, Figure 6).

In the animal allowed its customary dietary habit, epithelial sudanophilic inclusions are always granular and small.

The picture in the terminal ileum is more delicate - droplets being finer at each stage, and epithelial involvement less marked than those in the appropriate sections of jejunum.

Very few traces of absorption are seen in the ileum of animals kept on ordinary diet.

Sudan-tinting of cytoplasmic granularity in the luminal aspect of epithelial cells is noted again in caecum and distal colon. P.A.S. also gives a positive reaction in the granular supranuclear zone of the surface cells of these two regions.

3. Migratory Cells.

The cells are again granular leucocytes. Some show the bilobed nucleus. The granules are frequently basophilic or unstained and highly refractile. They do not always take up the Sudan dyes. Those showing the customary brownish-orange reaction with Sudan IV are so recorded. They give a positive reaction with P.A.S./

P.A.S. Direction of migration is similar to that seen in other species.

Very few are seen in any sections taken from the animals allowed their normal dietary habit, or from those fasted overnight. In those killed at 1½ and 3 hours they are still small in number but are slightly more numerous in the terminal ileum. (Guinea-pig. Plate 5, Figure 7).

They are increased in number in all sections of small intestine and caecum at 4½ hours. These numbers are maintained in duodenum and jejunum after 6 hours. Sections above and below these regions show only a few cells.

The most conspicuous feature of the guinea-pig material is the presence of the large, pale, mononuclear cells, each with granular cytoplasm and oval clear nucleus, seen in the stroma of mucosa and submucosa throughout much of the intestinal tract.

In the duodenum, jejunum and ileum they occur in groups of 7 or 8 cells situated together in the stroma at the tip of the villus, between the epithelium and the tip of the lacteal.

In/

In the caecum and large bowel similar groups occupy the lamina propria between the widely spaced crypts of Lieberkuhn.(Guinea-pig. Plate 6,Figures 8-10).

These cells are often frankly phagocytic and fragments of refractile material, resembling in colour reaction the red blood cells of the section, are seen within their cytoplasm. Occasional ingested granulocytes are detected. The other main contents, however, are frequently amorphous and unidentifiable and sometimes darkened with Sudan Black. At no time are deeply sudanophilic droplets resembling particles of absorbed fat seen in the cells.

In both small and large intestine they show a marked positive granularity with P.A.S.(Guinea-pig. Plate 6, Figure 8). Sections stained with Sudan Black or Sudan IV give a faint tinting to their cytoplasm. (Guinea-pig. Plate 6, Figures 9 & 10).

There is nothing in these sections to suggest that these cells undergo migration.

They also occur as isolated cells, infrequently placed, in some parts of the submucosa.

There is no variation in number, site or staining reaction at the various absorptive times.

4./

4. Regional Lymph Nodes.

Pyloro-duodenal, mesenteric and ileo-caecal nodes were examined.

In the first of these, traces only of free, Sudan-stained lymph are seen in the sinuses. Macrophages showing fine sudanophilic granules in their cytoplasm are most numerous at 3 hours. Those animals allowed normal dietary habit, show many such cells with varying amounts of P.A.S. positive granules in their cytoplasm.

There is little to suggest the passage of absorbed fat through the nodes from the ileo-caecal region.

Granulocytes, many of which show refractile, unstained granules with Sudan IV, are much more numerous in these guinea-pig nodes than in those from the other species examined. They are greatest in number at 3 and 4½ hours.

The mesenteric lymph node shows maximal extra-cellular, sudanophilic material at 3 hours, while both macrophage numbers, involvement, and droplet inclusion size reach their peak at 4½ hours. Considerable amounts of free material are still present in sinus tissue at this time.

4. GENERAL DISCUSSION.

1. Sites of Absorption.
2. Migratory Cells.
3. Macrophage Cells.
4. Lymph Vessels.
5. Regional Lymph Nodes.

GENERAL DISCUSSION.

The essential points which emerge from these descriptions of the gastro-intestinal tract during fat absorption are as follows:

1. Sites of Absorption.

While absorption of fat, as judged by the droplet inclusion size and number, and by the extent of the epithelial involvement, is probably maximal in the upper part of the intestinal tract at approximately 4½ hours, yet a considerable absorption of sudanophilic material may also occur over a much more extensive part of the tract, especially it would seem, in younger animals.

Stomach: The three zones of the stomach examined in the young ferrets are involved in such absorption at one phase or another. Thus fine sudanophilic granulation in a few surface cells of the fundic region at 3 and 4½ hours, and the body of stomach at 1½, 3 and 4½ hours (Ferret. Plate 4, Figures 5-6), give way to a considerable involvement of the mucus-secreting, columnar epithelium of the pyloric end of stomach, which is maximal from 3-4½ hours. This picture, which is quite distinct, presents only in the younger animals.

The/

The stomach in the fully mature animals remains completely negative.

Similar infranuclear, sudanophilic, granular inclusions are seen in some surface mucus-secreting columnar epithelial cells lining the body of the adult mouse stomach at 3 hours. (Mouse.Plate 4, Figure 8).

All sections from the adult hamster and guinea-pig stomachs remain negative to the Sudan staining.

An essentially different picture - that of Sudan-tinting - presents in the keratinised layers of the stratified squamous epithelium in the mouse and rat forestomach.(Mouse. Plates 1-2; Figures 1-5; Rat. Plate 1, Figures 1-2).

While much of the strongly Sudan-positive material seen adhering to the surface layers and carried into the upper layers by the knife edge, here and in the similarly lined oesophagus of the ferret, is undoubtedly due to ingested skin secretions, the pale, diffuse, but distinct Sudan colouration within the corneous layers themselves can hardly be regarded in this category. Such staining, though variable, is difficult to correlate with the post-ingestive phase concerned.

The/

The questions evoked by these findings would seem to be these.

In the mucus-secreting epithelium, what is the nature of the Sudan-stained inclusion material? Does it represent true absorption, in that it undergoes further transport to blood or lymph streams?

In view of the correlated medium-positive reaction with Periodic-Acid-Schiff, is the Sudan-staining in the cornified layers of the stratified squamous epithelium an expression of the nature of keratin itself, or does it represent the passage through it of some extraneous substance of dietary or other origin?

In how far is the picture affected by age, species and dietary habit?

Mendels & Baumann (1915) put forward the theory that histologically demonstrable fat passed into the epithelial cells of the stomach due to its preferential solubility in the cell lipoids, and that none passed through the submucosa into the blood or lymph streams.

In view of the fact that in the present investigation, fat was given to adult and young ferrets in the same/

same finely divided form, it is difficult to see why such preferential solubility should occur only in the cells of the young.

In common with Platt's (1954) findings in rats, the curd of ingested cream remained for long periods in the stomach of the ferret, and much of it was invariably present and filling the cavity of the organ, 4½ and even 6 hours after ingestion.

Platt (1954) has pointed out that, in the rat, only a small fraction of the fat separates out in the whey, and would therefore be expected to leave the stomach quickly. That remaining in the curd would be afforded a much longer opportunity for gastric lipolysis and absorption of its split products through the wall of the stomach itself.

Such an occurrence may explain why maximum 'loading' in the stomach lining cells of some animals frequently occurs long after the flood of absorption in the upper intestine has started, and also perhaps why it predominates in the young, where the more alkaline conditions prevailing would serve to facilitate the action of gastric lipase.

On the other hand, it is precisely in this animal, the/

the rat, in which stratification is known to occur and emptying of the stomach delayed, that no absorption of fat is found in this series.

This is contrary to the findings of Greene & Skaer (1911) who saw considerable absorption from the stomachs of old as well as young rats, and who associated the number of droplets in the cells with the length of time the fat had been in the stomach.

Platt (1954) has shown that the stomach contents in the rat pup after a breast feed taken on an empty stomach showed no stratification and more of a milk feed left an initially empty stomach quickly and without curdling than when added to material already present.

Under the conditions of the present experiments the non-physiological overnight fast before feeding may have resulted in this more rapid passage of the meal, and rendered minimal, opportunities for prolonged contact with the lining epithelium and for absorption from the body of the stomach of the rat. Some curds were present, however, in the stomachs of all animals examined.

Where present, it is of importance to note that, while the absorption picture is quite distinct, it in no/

no respect resembles that seen in the intestine, and attributed there to the presence of neutral fat.

The granulation in the stomach mucus-secreting epithelium tends to be highly refractile and a brownish-orange colour with Sudan IV. Individual particles are angular and not globular. In this respect the description seems to resemble that given by Jeker (1936) of the earlier stages of fat absorption in the small intestine of the rat and attributed by the lipolytic school, and indeed by Frazer himself (1946), to the presence of fatty acids in the cells concerned.

The absence of sudanophilic material from the stroma and lymph vessels of the wall of the stomach, seems to confirm the impression that the intracellular sudanophilic material represents fatty acids which pass directly to the blood stream, and which have resulted from some degree of gastric lipolysis.

The diffuse Sudan-tinting throughout the cornified layers of rat and mouse forestomach clearly does not represent the absorption of neutral fat. Its slight variability with species may be due to the inherent chemical nature and staining affinities of the type of keratin concerned, although some such tinting/

tinting with Sudan might be expected if fatty acids were in process of passage through it.

Fatty acids are known to give a pale yellow-orange colour with Sudan IV, and to show high refractility. They are also known to be able to pass through cornified epithelium.

The lining of the rumen of the sheep, like the skin, consists of stratified squamous epithelium of the keratinised type (Barcroft, 1944), but unlike the skin, the corneous layer is thin.(Barcroft, McAnally, Phillipson, 1943; Dobson, 1955).

Phillipson and his colleagues (1944-1953) have shown that certain lower fatty acids, resulting from cellulose fermentation, can pass through this epithelium, since their concentration in the veins draining the rumen is higher than that in the general circulation or in the blood leaving other parts of the gastro-intestinal tract.

Such absorption has also been shown to occur, though to a lesser extent, from the reticulum and omasum of the sheep, and from the non-ruminant stomach of the pig.

In/

In the light of these facts, there seem some grounds for suspecting that Sudan-tinting in the stratified epithelium may represent the absorption of certain lower fatty acids, of fermentative, or dietary origin.

Keratin, however, is known to contain phospholipides and cholesterol (Thorpe, 1947), in proportions which vary with the species concerned.

Pearse (1953) points out that some weak staining of phosphatides occurs with the Sudan dyes even in conventional techniques, and that if neutral fat is excluded, phosphatides are the commonest lipides demonstrated by Sudan Black.

This taken with the fact that phospholipides, but not neutral fat, are P.A.S. positive, is strong presumptive evidence that the weak Sudan-staning seen here, in areas which are also, in the sections so stained, P.A.S. positive, may be a measure of the phospholipide content of the keratin concerned.

Duodenum: The absorption picture, where present, in the epithelium of the first part of the duodenum, is one of sudanophilic granulation, and in this respect resembles/

resembles that seen in the mucus-secreting, columnar epithelium of the stomach.

The picture probably results from absorption of the products of gastric lipolysis.

It is seen only in the young ferrets (Ferret. Plate 5, Figure 7), and in some of the material from the adult rats and mice.

In the former, the granules are chiefly supra-nuclear, are present at 1½, 3 and 4½ hours, and though never gross in amount or distribution persist even in the 6 hour and fasting animals, chiefly in cells at the tips of the villi.

In the mouse material the inclusions are chiefly infranuclear, as in the stomach, and are seen in 3, 6 hour and fasting animals. (Mouse. Plate 5, Figures 9 & 10).

With the exception of Sudan-stained granularity in a few surface cells at 4½ hours, this area from the hamsters is negative. Equivalent areas from all guinea-pigs and adult ferrets show no sudanophilic material.

As with the stomach, stroma and lymphatics in almost all instances are negative.

Below/

Below the entrance of the bile duct, the typical globular inclusions attributed to the presence of particulate neutral fat, make their appearance.

Small Intestine: The fat absorption picture as seen from 1½ hours onwards in the herbivorous and omnivorous jejunum and ileum is too well known to need much elaboration here. It is sufficient to note that at any one time, droplet inclusions are not only maximal in the cells at the tips of the villi, graded downwards in size from tip to root of villus, and from luminal to basal aspect of the cell itself, but are greatest in size and number high in the jejunum. Even when marked absorption occurs in ileum, globules are always smaller and quite discrete, even in cells at the tips of the villi.

With reference to the mode of entry of fat globules to the cell, Wotten & Zwemer (1939) published illustrations showing an 'hour-glass' constriction in the individual droplet as it passed through the striae of the luminal border.

One illustration is presented here (Mouse. Plate 8, Figure 15) depicting a similar constriction, seen in side/

side view from the tip of a villus in the mouse jejunum. It is emphasised that such an appearance was seldom noted.

The very fine spindle-shaped particles described by Baker (1951) as lying completely within the canals of the free border are not demonstrated by the traditional techniques of the present study.

Active absorption is extensively in progress in all animals at 1½ hours, and is maximal around 4½ hours.

In the mouse and hamster, the upper jejunum is never completely negative - residual fine droplets persisting in the infranuclear position of some cells even after overnight fasting.(Mouse. Plates 7-10, Figures 13-22).

In the lower jejunum and in those animals where only the mid-jejunum was examined, fasting specimens are negative and the absorption picture at 1½ hours delicately fine. (Mouse. Plate 11, Figure 23).

Free material in stroma and lymphatics confirms the route of absorption. (Mouse.Plate 11, Figure 24).

Ileum: The fat absorption picture as seen in terminal/

terminal ileum is of fine discrete globules, and begins in the mouse in the 4½ hour sections.

In hamster and rat it would appear that, of the fat administered, almost all has undergone absorption before reaching the lower part of the small bowel. Only slight sudanophilic granulation is to be seen in the cells at the tips of some villi at 3 hours.

Only in the guinea-pig are sudanophilic droplets, fine and discrete, noted in this area at 1½ hours (and in subsequent ~~hours~~ sections). Frequently, in both jejunum and ileum, where maximum epithelial involvement is seen at 4½ hours, individual droplets of larger size are noted in cells at tips of villi in 6 hour sections.

As will be noted from the tables, absorption of fat, in the essentially different type of gut of the ferret, is active far down the tract in almost all sections.

Caecum: In the mouse, fine sudanophilic and chiefly infranuclear granules, while present in groups of the surface cells in all sections examined, tend to be minimal in number and extent of epithelial involvement/

ment at 1½ hours (Mouse. Plate 12, Figure 26), and 3 hours, and to increase in the later phases of absorption at 4½ and 6 hours (Mouse. Plate 13, Figures 27 & 28). Here some approximate to half the nuclear size. 12 hour, and overnight fasted animals show considerable involvement (Mouse. Plate 12, Figure 25).

Only pale Sudan-tinted granularity of the supra-nuclear cytoplasm is noted in the hamster and rat material. In this respect the cytoplasmic staining is only slightly deeper than that of the other tissues in the sections.

Similar, but slightly more marked, supranuclear yellowish granularity is seen with Sudan IV in the surface epithelium of the guinea-pig caecum. In guinea-pig and rat, this granular, supranuclear zone is positive with P.A.S.

These two pictures are essentially different - that in the mouse resembles the absorption picture of granules (probably of fatty acid) seen in the body of stomach; while that in the guinea-pig, rat and hamster are diffuse cytoplasmic stains.

In all but the guinea-pig, where nests of large phagocytic/

phagocytic cells, containing Sudan and P.A.S. positive inclusion material, are seen in the lamina propria between crypts (Guinea-pig. Plate 6, Figures 8-10), stroma and lymphatics remain negative to the s tain.

There is no caecum or equivalent region in the ferret intestinal tract.

From this series of observations it would appear that only in the mouse did any degree of real absorption take place from this part of the intestine. The distinctly Sudan-stained picture, though never gross, and appearing at its greatest as it did in the later stages after ingestion, would suggest that residual fat, or its split products, reaching the caecum, can be absorbed by this absorptive striate-bordered epithelium, even in the absence of villi.

The failure to trace fat-stained material in stroma or lymphatics, together with the often angular shape of the granular inclusions, is strongly indicative of the absorption of fatty acids.

The diffuse tinted granularity of lining cells in all other animals may be a measure of their content of phospholipide which is known to stain weakly with Sudan even in the conventional techniques, and gives a distinct/

distinct result with the P.A.S. method.

As with the ruminant stomach, however, Barcroft, McAnally & Phillipson (1943,1944,1947) found cellulose fermentation with absorption of resulting volatile acids in the large intestine of the horse, pig, rabbit, sheep, rat and dog. In the rabbit these fatty acids were absorbed about three times as rapidly from the caecum as from the small intestine. In the event of such decomposition and absorption occurring in the caecum and large intestine of other herbivores one would expect some indication of this in sections stained with Sudan, and indeed examination of material taken from a rabbit caecum confirms that a distinct and similar cytoplasmic staining with Sudan dyes occurs, especially in the luminal aspect of the surface cells.

Proximal Colon:

Minute, discrete and distinctly sudanophilic granules are seen, chiefly in the infranuclear position, in some surface cells of the mouse proximal colon at 3 and 4½ hours. (Mouse. Plate 14, Figure 30).

This finding is in contradiction to those of Nakashima (1914) who found no absorption after rectal administration/

administration of milk. Here it almost certainly represents, however, some degree of absorption since it resembles the stomach absorption picture, etc., The substance concerned is probably fatty acid, since stroma and lymph vessels remain negative to the stain.

Since Nakashima separated small from large intestine before experimentation, it may be that he obtained a negative result by thus obviating the possibility of lipolysis.

Only a very pale, diffuse, cytoplasmic Sudan-tinted granularity is detected in supranuclear cytoplasm of surface cells in all sections from the hamster, guinea-pig, much of the rat material, and in the sections from the second last portion from the adult ferret intestine.

Again this may be an expression of the staining affinities of the cytoplasm itself, and of its phospholipide content.

The facts of endogenous fat excretion and reabsorption, and of fatty acid absorption from the caecum and colon, however, cannot be ignored.

The Sudan IV dye is soluble, in vitro, in Phillipson's/

Phillipson's lower fatty acids.

Histological examination of the caecum and proximal colon in the rabbit confirms that diffuse Sudan-staining similar to that seen in rat, hamster, guinea-pig, and adult ferret, does occur in the upper part of the surface cells.

Apart from this question of fatty acid absorption resulting from cellulose fermentation, faeces are known to contain fatty material consisting mainly of fatty acids, and their soaps, smaller amounts of cholesterol and its derivatives, and a little neutral fat (Bloor, 1922). This material is little affected by the fat of the food (Holmes & Kerr, 1923; Angevine, 1929).

Sperry and his colleagues (1932) showed that this represented a secretion into the intestinal lumen of lipid material essentially similar to depot and plasma fat.

Their investigations showed that the excretion originated principally in the small intestine. Their finding that much larger amounts of lipids were excreted from fistulas of the ileum, in dogs, than were excreted in the faeces on a fat-free diet, forced the/

the conclusion that considerable absorption of lipids occurred from the colon.

It is suggested that some correlation between these facts and the histological picture is to be expected.

Only in the carnivorous ferret is the absorption picture, as seen in the lower portions of the intestine, likely to be of dietary origin.

In the ferret, short villi persist to within 7 centimetres of the bowel's termination,(Ferret, Plate 10, Figure 16), and in this portion in the young animals, considerable involvement of the surface cells with fine, supranuclear, sudanophilic granules, is seen at 1½ hours and in subsequent sections. Maximal epithelial involvement and droplet inclusion size are noted at 4½ hours, and some granules penetrate to the infranuclear zone at 4½ and 6 hours.

Traces of Sudan-stained material are seen in submucosal lymph vessels in this villous part of the ferret tract, and also in its local lymph node.(Ferret. Plate 16, Figures 27 & 28).

Distal Colon:

The/

The distal colon near its termination remains negative to the fat stains in all rat and guinea-pig material.

Faint but distinct Sudan-tinted granularity is visible in the lining cells of the mouse, hamster and adult ferret terminal colon.

Stroma and lymph vessels show no Sudan-staining.

Only in the surface cells in the young ferret again are discrete, sudanophilic droplets seen which vary in number and distribution with the post-ingestive phase.(Ferret. Plate 12, Figures 19-21).

These droplets which are small and supranuclear, are accompanied by infranuclear sudanophilic granulation at 3, 4½ and 6 hours. They are maximal in number and size at 4½ hours,(Ferret. Plate 12, Figure 21), but though less in amount ,there is still considerable epithelial involvement after 6 hours, which persists in the fasting (Ferret. Plate 12, Figure 19), 1½ (Ferret. Plate 12, Figure 20) and 3 hour sections.

Though villi are absent and no free material or positive staining lacteals can be seen in the stroma between crypts, one or two submucosal lymph vessels are detected showing the stain at 1½ and 3 hours, and

a/

a faint trace of free sudanophilic material with distinct Sudan-stained macrophages are seen in the local lymph node.

Only in the young ferret, therefore, can the histological picture in the terminal colon be interpreted as due to the absorption of fatty substances probably of dietary origin. In general shape and colour reaction, the sudanophilic inclusions resemble those seen in the villus-bearing part of the tract.

It is obvious that in the ferret, the range of absorbability in the intestine is much greater than that seen in the other animals investigated.

Even in the carnivorous ferret, however, where large amounts of finely emulsified fat are normally ingested, and with its relatively short intestinal tract, minimal dietary roughage, and relatively rapid intestinal emptying time, the possibility of absorption of fat, from the non-villous part of the tract, seems limited to the younger animal.

2. Migratory Cells.

(a) Nature and Staining Reactions.

In the past there has been considerable discrepancy in the interpretation of results regarding the nature of these cells, and a complete failure to differentiate adequately the cell types involved.

In all sections examined here they are undoubtedly granular leucocytes. Further classification is difficult since paraffin embedded material stained by haemalum and eosin, while showing clearly the eosinophilic cytoplasm and the basophilic kidney-shaped, horse-shoe or ring forms of the nucleus, fails to demonstrate the granular nature of these cells.

This is a well-recognised finding. Bunting (1922,1938) showed that in man there were polymorphonuclear leucocytes with a neutrophilic staining reaction. The heterophil leucocyte of the laboratory animals, while resembling the human neutrophil quite closely in size and morphology, showed slight variation in nuclei and in size and staining of the specific granules which made them distinctive. In the rabbit this morphological and functioning equivalent cell had an eosinophilic reaction, while in the guinea-pig this cell/

cell was amphophilic. In the rat and mouse the granules were undemonstrable by use of the ordinary stains.

The sudanophilic element is the granule. These granules vary in number and size in the various species examined, being much finer in the leucocytes of the hamster, rat and guinea-pig, and coarser in those of the ferret and mouse.

The staining reaction likewise varies. Fewer are noted with distinctly sudanophilic granules in the tissues of the guinea-pig gut and lymph nodes. Here the granules are frequently basophilic or dark and highly refractile, taking the counter-stain instead of the Sudan dye.

In all other cases they stain with Sudan IV and Sudan Black. With the former stain the granules show a brownish-orange or brownish-yellow colour reaction.

Sections from rat and guinea-pig which were stained by P.A.S. show positive granules.

In only a few instances, and these chiefly in the/

the large bowel, are they prominent with Sudan Black, and indistinguishable with Sudan IV.

Lison (1936), when first introducing Sudan Black as a general fat stain, noted that it was soluble in all or nearly all classes of fat, and in fats only.

The leucocyte granules have customarily been regarded as protein in nature.

Bacsich (1935) found lipoid granules, as seen in Sudan III staining, to be a constant physiological constituent of the human blood granulocytes, and the appearance and arrangement of these granules in the various leucocytes was identical (except for colour) with those of the granules stained by the customary blood staining methods or by the oxydase reaction.

McManus (1945), describing a method for staining them in human blood granulocytes, found that while 2% of his polymorphonuclear leucocytes contained fat with Sudan IV (and this was taken by him as an indication of aging), all stained with Sudan Black. This suggested to him that they were 'lipine', or contained 'lipine'. Their insolubility in acetone strongly supported the idea, though by no means proving it, that they were lecithin.

He, however, found none of these granules in cells from lymph node punctures.

Both Bacsich & McManus found that in human blood leucocytes the number and size of the lipoid granules did not alter after fasting or feeding.

Sheehan (1939) also showed that polymorphs, particularly neutrophils, in Sudan Black sections, were filled with deeply stained granules, as distinct from those in the eosinophils which appeared to have only a surface layer of lipoid.

In view of these findings there is a certain justification for concluding that the majority of the cells seen in histological sections from the intestinal wall are probably of the neutrophil (or heterophil) class.

Regarding the exact chemical nature of this lipoid, little is known, but it is established (Boyd, 1935), that the white blood cells of blood have large amounts of phospholipids, and in view of the P.A.S. reaction as well as the Sudan staining, it may be that much of this reposes in the granular element of these cells.

(b) Site of Origin.

Schäfer/

Schäfer (1912) believed that the 'amoeboid cells' had their origin in the lymphoid tissue of the gut wall. He denied that they could have their origin in blood vessels. Part of his difficulty arose from a failure to differentiate the various white cell types.

Hardy & Wesbrook (1895) mention vaguely their origin and migration 'from the base of the mucosa'.

It seems obvious from their presence, even in the relatively quiescent phase of their activity, in and near blood vessels of the submucosa (e.g. Rat. Plate 1, Figure 1) and those at the base of the mucosa, that they migrate outwards from such vessels into the tissues of the mucosa in response to some stimulus as yet undetermined.

(c) Direction of Migration.

The occasional presence, in all animals examined, of these cells in the luminal debris and in the act of migration through the epithelium seems to confirm the direction of their migration (e.g. Hamster, Plate 1, Figures 1 & 2).

(d) Distribution in the Tissues of the Intestinal Wall and Relationship to Absorption of Fat.

From/

From a thorough survey of the literature and the observations reported here (in the separate summaries for each species), it is concluded that no constant relationship can be traced between the numbers or invasion of the intestinal mucosa by these cells, and the processes of absorption.

Heidenhain (1884) & Leach (1938) found that 'eosinophils' often decreased in number during digestion.

Here, in almost all instances, the heterophils show a tendency to increase during the absorptive phase, reaching a peak frequently between 4½ and 6 hours. In general they are probably maximal in numbers in sections taken from approximately mid- or lower jejunum, and diminish as sections pass from this towards either stomach or colon.

Exceptions, however, occur, and occasionally, as in the rat, the 'wave' of these cells appears to be synchronised throughout the tissues of the whole tract, or, as in the mouse, larger numbers may be seen in the wall of the large intestine.

(e) Function.

The harder problem to solve is the physiological purpose of this migration.

The/

The above observations, taken with the experimental findings cited, support the conception that the migratory leucocytes of the gut wall, like those of the blood stream, can play no part in the transport of absorbed fat or indeed in the processes of absorption at all.

Their overwhelming numbers in all tissues of the rat which, after 22 hours' fast, was subjected to a stomach wash-out, raise the question as to whether stress, distension, or the invasion of the intestine by micro-organisms, play any part in the stimulus to migration.

Posture is known to affect the blood leucocyte count (Garrey & Butler, 1932). These workers found that sudden distension of the human stomach, or abrupt changes in gastric temperature, due to hot or cold fluids, gave an immediate, though mild and transient rise in the blood leucocyte count. Animal experiments indicated that this type of reaction was due solely to reflex vascular disturbances and bore no relationship to absorption.

There is the possibility that such a factor is at work here.

The function subserved by this process of extrusion of/

of leucocytes is still very much in the realm of conjecture.

At this stage it is still impossible to do more than point out a few provocative facts and suggestions.

Sabin (1925) pointed out that the total white blood cells of the blood increased in the afternoon, irrespective of food, and that this entire increase was the result of an increase in neutrophilic leucocytes.

The neutrophil leucocytes die out 'in showers', often of considerable proportions. These dead cells are promptly replaced.

These two facts, taken with McManus's opinion that leucocytes which stain with Sudan IV show signs of aging, leads one to enquire whether this process of extrusion and disintegration within the gut lumen, is simply a way of getting rid of aging leucocytes, especially in view of the finding that some undergo phagocytosis by macrophage cells during the process.

One further possibility suggests itself.

Representing as it probably does, a more or less continuous process of extrusion, with consequent disintegration/

disintegration and release of their contained lipide granules, it is suggested that these cells may account for a considerable proportion of the endogenous fat excretion.

Such a possibility was glimpsed by Schönheimer & von Behring in their communication to Sperry & Angevine (1932), when they drew attention to 'the large number of lymphocytes in the intestinal lumen', and suggested that the lipids found in the intestinal excretion might be, to a large extent, contained in these cells. In view of more recent findings, the cell type involved is more likely to be the heterophil leucocyte with its integral lipoid granules.

Their preponderance in the wall of the small intestine, coupled with the definite but smaller numbers in the wall of the large intestine, accords with the finding that the larger proportion of the endogenous fat excretion arises from the small intestine, and that a smaller amount has its origin in the colon.

3. Macrophage Cells in Gut Wall.

Certain other large mononuclear cells, many times the size of the migratory granulocytes, each with one eccentrically/

eccentrically placed nucleus showing scanty chromatin, are a prominent feature of the mucosa of the guinea-pig small and large intestine.

These large phagocytic cells form a subepithelial cap to the lacteal of the villus in the small intestine, and foci, each of 5 to 8 cells, in the stroma between the widely placed crypts of the large intestine. Such cells are absent, inconspicuous or infrequent, in the other species examined. Occasional members are seen in the submucosa.

They are particularly prominent in the caecum and large bowel.

They give a positive reaction with P.A.S. and a weak colouring of their cytoplasmic granularity with the conventional Sudan stains.(Guinea-pig. Plate 6, Figures 8-10).

They resemble markedly the free macrophages of reticulo-endothelial origin seen in the sinus tissue of the mesenteric lymph nodes, but Sudan-stained inclusions, which are discrete and globular in the latter, tend to be granular and much less easily identified in the macrophage cells of the gut wall. Inclusions/

Inclusions are often amorphous, highly refractile and virtually unstained. In this they resemble broken down red blood cells. Although the cytoplasm is palely tinted with the Sudan stains, only when effete granulocytes (whole or broken down) are included, is any marked staining seen with Sudan dyes and then only in the integral granules of the leucocytes.

In the hamster, isolated free macrophages are seen in submucosa and especially at the base of the mucosa.

It seems that these are the cells described in the guinea-pig by Macallum (1894) as responsible for the absorption of iron..... 'leucocytes, which in their disposition, form a cap as it were for the extreme end of the lacteal vessel'. The dark colour in his sections due to the formation of sulphide of iron was limited to the 'subepithelial portions of the tips of the villi'.

His accompanying diagrams, however, do not fit these cells so well as his description, and it is obvious that, along with many of the older writers, he showed considerable confusion in the recognition and classification of the cell types involved.

Many/

Many described by Hardy & Wesbrook (1895) as 'phagocytic', in epithelium and stroma, are lymphocytes, though they did describe 'Hyaline Cells' which displayed ingested fragments in their cytoplasm.

Leach (1938) described subepithelial cells, 'probably histiocytic' and 'perhaps containing fat' in the intestine of the rabbit. He thought that these cells migrated through the epithelium to disintegrate in the gut lumen. Any free fat particles ingested from the stroma were purely incidental and of no importance in the purposive absorption of fat. (Indeed such fat represented a slight loss in efficiency in fat absorption since it was carried back across the gut wall in the cell's migration.)

There is nothing in the sections examined here to suggest that such migration through the gut epithelium occurs. The cells stay grouped in the core of the lamina propria in material from each of the post-ingestive phases investigated. They coexist in the stroma with granulocytes, many of which show Sudan-stained granules. No periodicity or notable differences in distribution or numbers could be detected at the various times.

It is suggested that these are macrophages of reticulo-endothelial origin; that their main function in the tissues is as 'scavengers'; that they rarely, if ever, ingest fat, and that the P.A.S. positive reaction, together with the Sudan-tinting of their granular cytoplasm, may be a measure of their pigmented nature - the pigment concerned being a lipofuscin, which is known to react to these stains in this way (Pearse, 1953).

4. Lymph Vessels.

It is to be stressed that in neither the lymph vessels of the gut wall, nor those leading in to the regional lymph nodes, are any cells (phagocytic or granulocyte) seen intraluminally or within the wall of the vessels in any of the species examined.

In this respect the findings of other writers, such as Leach (1938), are not confirmed.

It has already been indicated that the repeated finding of Sudan-stained lymph in the submucosal lymph vessels in the wall of the gut in parts where no absorption occurred through the surface epithelium, seems/

seems to confirm the impression that these vessels (of the submucosal network) are in longitudinal continuity, and that spread of lymph with its contained products of absorption, is thus made possible within it.

5. Regional Lymph Nodes.

In many of the mesenteric lymph nodes, especially in those from the young ferrets, it appears that most of the Sudan-stained extracellular material reaching the nodes from the intestinal lacteals, is phagocytosed before reaching the inner medulla.

As distinct from this picture in the large regional nodes, much more free material apparently traverses small local nodes, such as those adjacent to the gut wall, without the intervention of these macrophage cells, and presumably is subsequently transported to the regional node for this process.

No cells of this, or the granulocyte class, are seen in any afferent lymphatics, and one is forced to conclude that the macrophages surrender their fat content to the efferent lymph vessels in the free state.

It may be, however, that these cells, by some pathway/

pathway not determined, are destined for the mesenteric fat depots and that only such phagocytosed fat passes to these local depots, while that escaping the intervention of the cells proceeds to the systemic blood.

Further histological investigation of the thoracic duct lymph might yield information in this respect.

The macrophage cells increase in number and Sudan-droplet involvement as absorption proceeds, and show maximal numbers in most animals somewhere around 4½ to 6 hours. (Ferret. Plate 13, Figures 22 & 23). In the later stages, droplet inclusions, which have been increasing in size at first, become much finer. In other words, it would appear that some form of intracellular breakdown is occurring within these cells. As intracellular fat increases, extracellular fat (in sinus tissue) diminishes.

In the guinea-pig, in which Frazer (1945) found little evidence (in chylomicron counts, etc.) of fat absorption similar to that found in other species, sinus tissue throughout the node at 3 hours shows free, sudanophilic material apparently destined for the systemic blood. Although macrophages are numerous and phagocytosis active, much of this free material/

material appears to escape intracellular ingestion.

One other point emerges from the comparative study of the lymph nodes.

In the node from the wall of the young ferret intestine - Portion F - the presence of free sudanophilic material is doubtful, yet macrophage cells with Sudan-stained globules increase markedly in number and involvement at 3 and 4½ hours, confirming the impression that fat of dietary origin is in process of absorption in this terminal portion of the colon.

It appears that in all species examined, occasional granulocytes wander through the walls of hilar blood vessels and invade the tissues of the nodes.

This process seems unrelated to the prevailing absorptive phase, though it is suspected that it is in some way related to the stimulus which effects migration in the wall of the gut, since, in the 22-hour fasted rat especially, where all tissues of the gut wall are flooded by these cells, lymph nodes show much greater numbers of the cells than those from any of the other animals examined.

SUMMARY.

SUMMARY.

The Histology of Fat Absorption, with Special Reference to the Sites of Absorption, the Migratory Cells, and the Regional Lymph Nodes.

Past work on the histology of fat absorption, together with leading experimental and biochemical views, is reviewed.

A comparative histological study of fat absorption, as seen in the gastro-intestinal tract and its regional lymph nodes at various absorptive stages after the ingestion of 'skim-cream', has been carried out in ferrets, mice, hamsters, rats and guinea-pigs. The detailed histological findings in sections stained with Sudan stains, and occasionally with Periodic-Acid-Schiff, are presented in tabular form.

The essential points which emerge are as follows:

1. Sites of Absorption.

Maximal absorption of sudanophilic globules occurs in the upper part of the small intestine at approximately/

approximately 4½ hours. The picture suggests particulate absorption of fat. Attention is focussed on the granular sudanophilic material in the mucus-secreting columnar epithelium of the stomach in the young ferret and adult mouse, and in the first part of the duodenum. It is suggested that this represents fatty acid of dietary origin - gastric lipolysis being facilitated by the curd's prolonged stay in the stomach.

Similar absorption is seen in the caecum and proximal colon of the adult mouse.

In the ferret, short villi persist to within 7 centimetres of the gut termination. A fat absorption picture similar to that seen in the small intestine, is noted in this and in the terminal non-villous part of the tract in the young animal.

Diffuse Sudan-staining in the Stratum Corneum of the stratified squamous epithelium lining the fore-stomach of the rat and mouse, and in the supranuclear cytoplasm of the epithelial cells in the caecum, the proximal, and occasionally in the distal colon of the rat, hamster, guinea-pig and adult ferret, may be a measure of the phospholipide content of the cells concerned, though the possibility of the presence of fatty/

fatty acids resulting from cellulose fermentation, and, in the case of the large bowel, the reabsorption of endogenous fat excreted higher in the ileum, cannot be excluded.

2. Migratory Cells.

The Sudan-stained migratory cells of the gut wall are granular leucocytes of the heterophil class. The integral granules are the sudanophilic element. In the guinea-pig the Sudan response is variable. A positive reaction is obtained with Periodic-Acid-Schiff.

The cells migrate from blood vessels in the base of the mucosa and the submucosa; they eventually disintegrate in the lumen of the gut. The stimulus to migration is undetermined, and no constant relationship is detected between the degree of mucosal invasion and the absorptive phase. The 'wave' of cells is frequently synchronised throughout the whole gut wall, and the influence in this of distension, stress, or intestinal invasion by micro-organisms, is suspected.

The cells play no part in the transport of absorbed fat. It is suggested, however, that in the continuous process of their extrusion and disintegration they/

they may rid the body of aging leucocytes. The consequent release of contained lipides may account for part of the endogenous fat excretion.

3. Macrophage Cells.

Macrophage cells, apparently of reticulo-endothelial origin, are a prominent feature of the mucosa in the guinea-pig, forming a subepithelial cap to the lacteal of the villus of the small intestine, and foci, each of 5 to 8 cells, in the stroma between the widely placed crypts of the colon. Such cells are absent, inconspicuous or infrequent in the other species examined. Occasional members occur in the submucosa.

Their chief function is apparently the phagocytosis of effete cells. Other amorphous and unidentified inclusions are noted. There is nothing to suggest the ingestion of fat, or the migration of these cells through the gut epithelium.

Their staining reaction with Sudan and P.A.S. may indicate the presence of a Lipofuscin pigment.

4. Lymph Vessels.

Submucosal/

Submucosal lymph vessels appear to be in longitudinal continuity, thus facilitating spread of lymph with its products of absorption, within the network.

At no time are cells (macrophages or granulocytes) seen within the lumen of lymphatics of the gut wall.

5. Regional Lymph Nodes.

Much of the Sudan-stained lymph is ingested by reticulo-endothelial macrophages within the nodes, and is apparently subjected to some form of intracellular breakdown.

Maximal numbers and involvement of macrophages occur about 4½ to 6 hours after ingestion of fat.

Sudan-stained granulocytes migrate from hilar blood vessels into the tissues of the nodes. There is evidence to suggest that, though unrelated to the process of fat absorption, migration is effected by those stimuli causing invasion of the gut wall itself.

The herbivorous guinea-pig's lymph nodes show an essentially similar picture to those from the other species.

ACKNOWLEDGMENTS

Acknowledgments.

I acknowledge gratefully my indebtedness to Professor R.C.Garry for initial opportunities and continued, encouraging interest; and to Professor S.Alstead, who graciously afforded facilities for working in his own busy department.

I would express my thanks to Mr. T. Gorrie, who has been ever ready to assist in surmounting the technical difficulties encountered; and to the members of his staff - Mr. D. MacAllister for the preparation of the photomicrographs; and Miss Anna McCaffery and Miss Rita McPhail who were largely responsible for the care of the experimental animals, and the sectioning and staining of the histological material.

I am also indebted to Dr. J.G.Gillespie for help in translation of the German authors, and to Mr. R. Callander for the diagram in Volume II.

My especial debt is to Dr. H.S.Garven, in whose department it has been a high privilege to serve. He has been at all times generous in giving help and patient with the encroachments made on his time.

The cost of the research has been defrayed in part by grants from the Rankin Memorial Trust Fund.

THE HISTOLOGY OF FAT ABSORPTION WITH SPECIAL REFERENCE TO THE SITES OF ABSORPTION, THE MIGRATORY CELLS AND THE REGIONAL LYMPH NODES.

A THESIS submitted to the Faculty of Medicine in candidature for the Degree of Doctor of Philosophy.

by

ANN B McNAUGHT.

The Institute of Physiology,
The University of Glasgow.

September, 1955.

VOLUME TWO.

CONTENTS.

CONTENTS.

VOLUME TWO.

ii. Mouse

Plate	1	Forestomach	1-2
	2		3-5
	3		6-7
	4	Body of Stomach	8
	5	Duodenum - First Part	9-10
	6		11-12
	7	Jejunum - Upper Third	13-14
	8		15-17
	9		18-20
	10		21-22
	11	Lower Third	23-24
	12	Caecum	25-26
	13		27-28
	14	Proximal Colon	29-30
	15	Distal Colon	31

iii. Hamster.

Plate	1	Caecum	1
		Proximal Colon	2

iv. Rat

Plate	1	Forestomach	1-2
	2	Jejunum	3
	3	Proximal Colon	4

v. Guinea-pig

Plate	1	Duodenum - First Part	1-2
	2	Jejunum - Upper	3
	3	- Mid	4-5
	4		6
	5	Terminal Ileum	7
	6	Caecum	8-10

Bibliography.

INTRODUCTION

INTRODUCTION.

The series of illustrative photo-micrographs, the diagram of the ferret gastro-intestinal tract, and the Bibliography, are presented, for the reader's convenience, in this separate volume.

PLATES

FERRET.

PLATES 1 - 16.

(FIGURES 1 - 28)

FERRET : PLATE 1.

Diagram of Gastro-
Intestinal Tract in
Adult Ferret.

(Areas taken for histological
examination are outlined).

FERRET

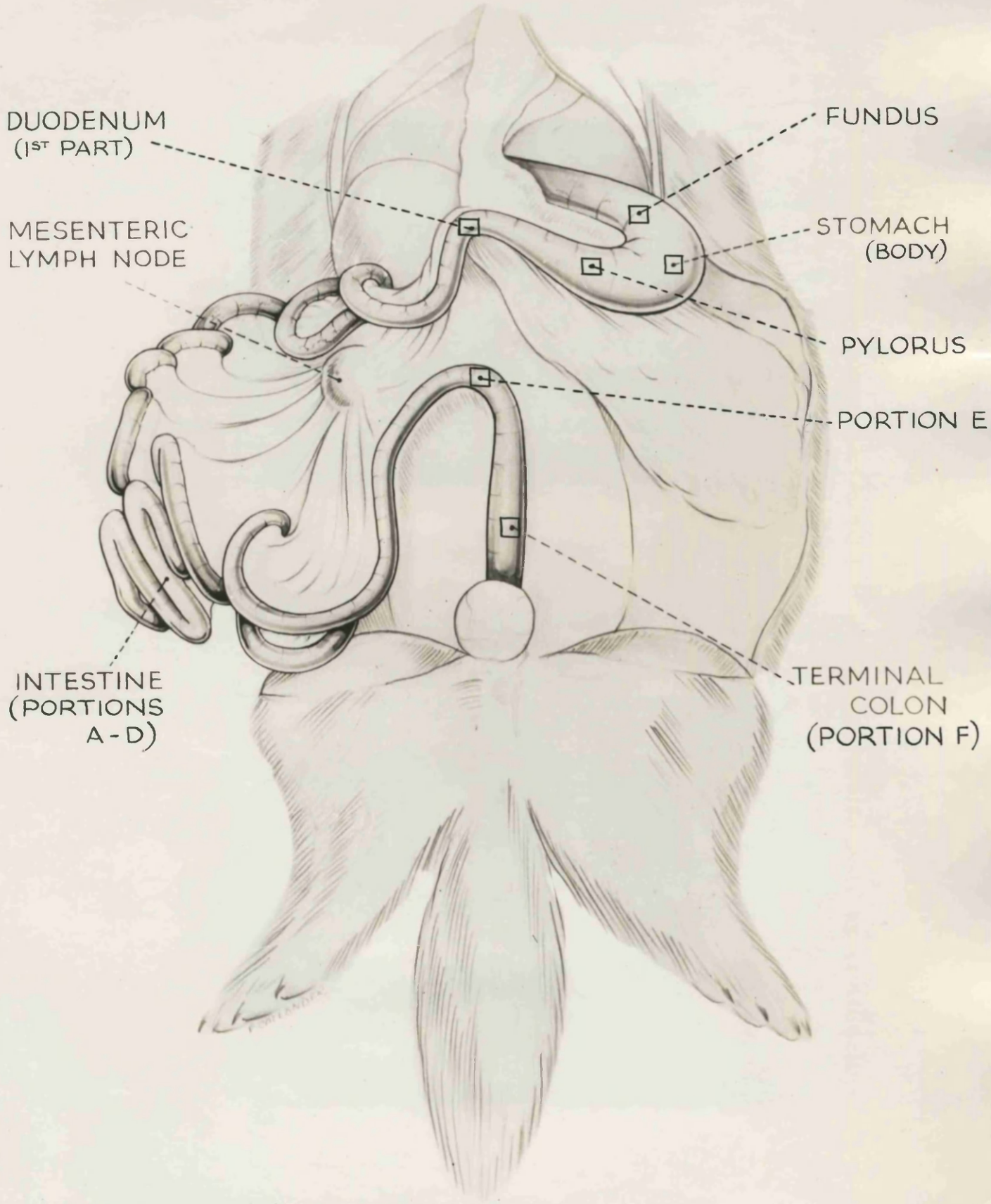


PLATE 1.

FERRET : PLATE 2 : FIGURES 1 & 2.

The Oesophagus.

Figure 1: Young Ferret Oesophagus (Upper Third) Fasting.
Sudan IV with Haemalum.
Magnification x 600.

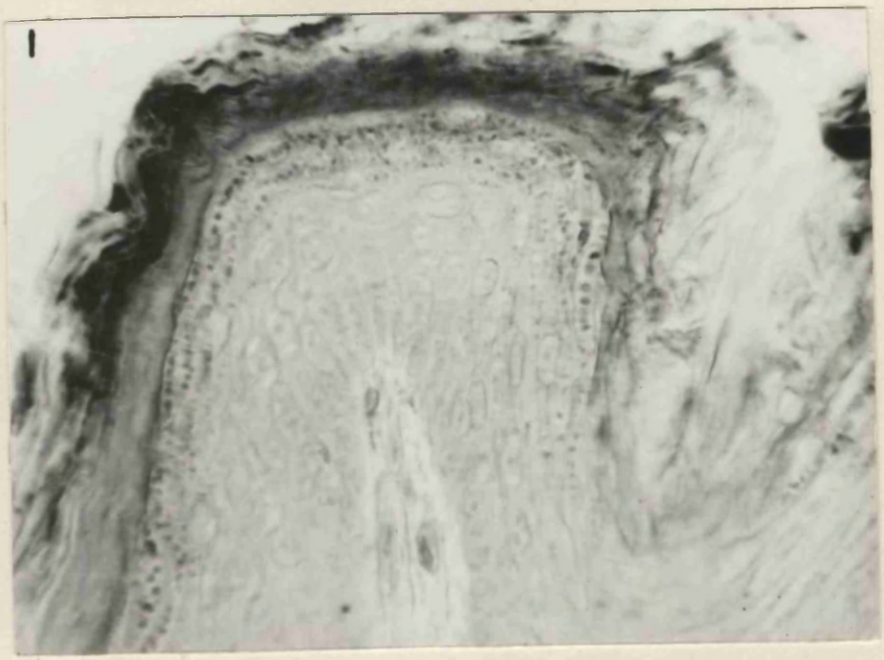
Stratified squamous epithelium with cornified surface layers. Prominent basophilic granules of stratum granulosum. Sudan-stained droplets and tinting in the stratum corneum.

Figure 2: Young Ferret Oesophagus (Upper Third) 1½ hours after feeding with 50 c.c. 'skim-cream'.
Sudan IV with Haemalum.
Magnification x 150.

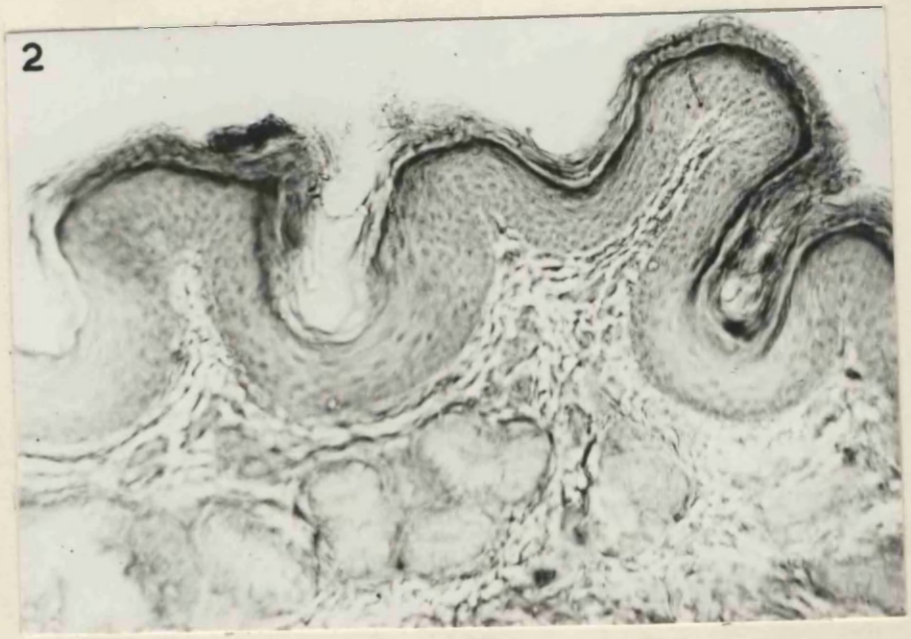
Some sudanophilic material in the upper cornified layers. Stratum granulosum is sharply picked out by the haemalum counter-stain. (Occasional granulocytes in the lamina propria immediately deep to the basement membrane of lining epithelium).

FERRET PLATE 2 FIGURES 1 & 2

OESOPHAGUS



X 600



X 150

FERRET : PLATE 3 : FIGURES 3 - 4.

Stomach.

Figure 3: Adult Ferret Stomach (Fundus).
4 hours after 'skim-cream'.
Sudan IV with Haemalum.(Aortic Perfusion)
Magnification x 600.

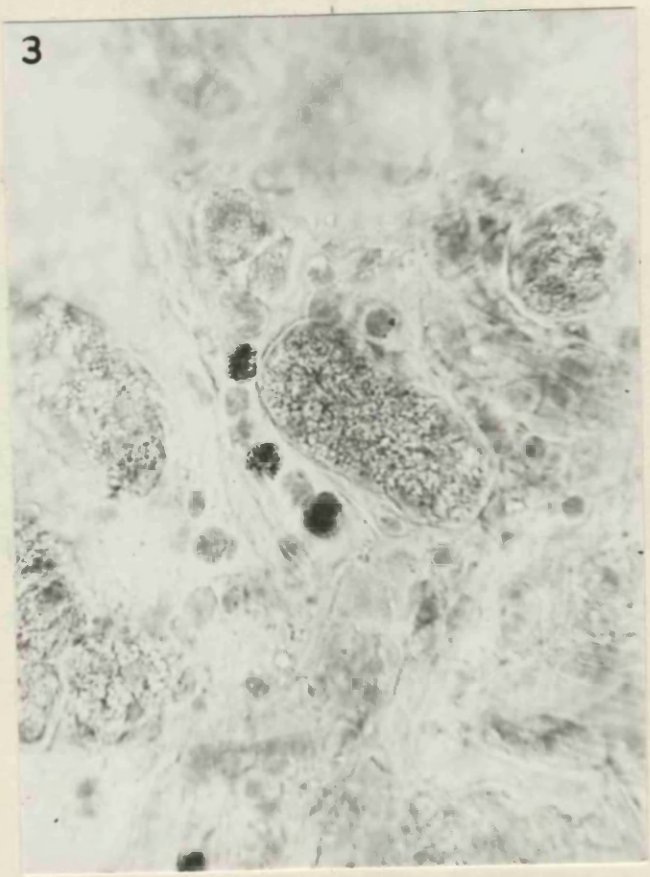
Migratory granulocytes, with Sudan-stained granules, round base of test-tube glands.

Figure 4: Young Ferret Stomach (Cardia).
3 hours after 'skim-cream'.
Sudan IV with Haemalum.
Magnification x 80.

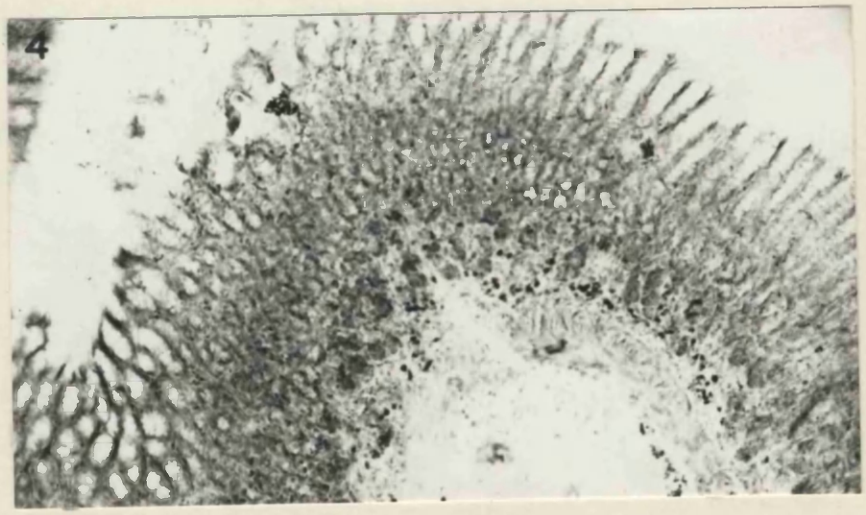
Migratory cells with Sudan-stained granules round base of test-tube glands, and in submucosa.

FERRET PLATE 3 FIGURES 3 & 4

STOMACH



X 600



X 80

FERRET : PLATE 4 : FIGURES 5 - 6

Stomach.

Figure 5: Young Ferret Stomach (Body of Stomach).
3 hours after 'skim-cream'.
Sudan Black.
Magnification x 80.

Fine, supranuclear, sudanophilic granulation in surface epithelial cells, deep to the u-shaped mucus-secreting part of the cell.

Oxyntic cells are also darkened by the stain.

(Blood vessels are dark in the photographic print).

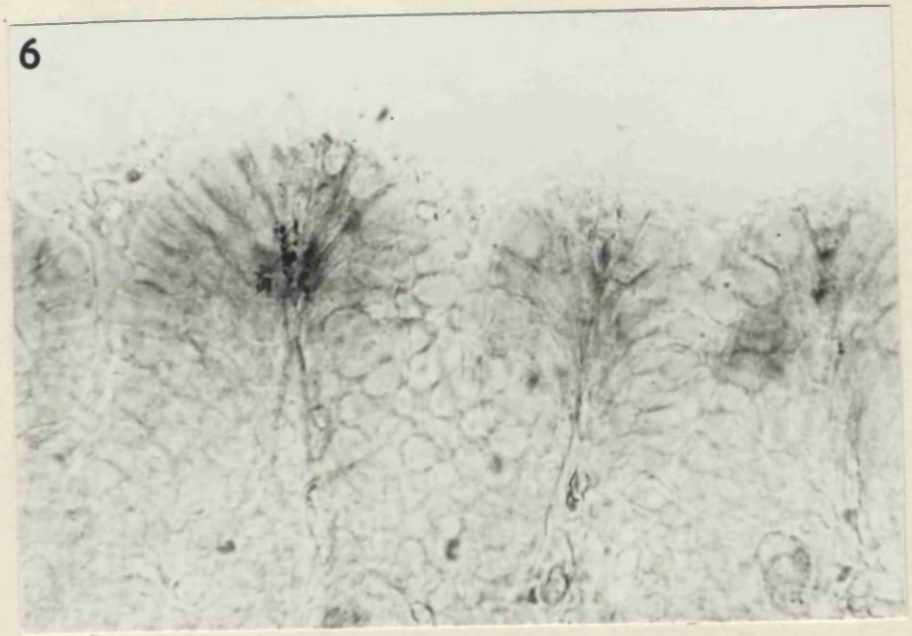
Figure 6: Young Ferret Stomach (Body of Stomach).
1½ hours after 'skim-cream'.
Sudan Black.
Magnification x 600.

Fine sudanophilic granules in infranuclear position of surface mucus-secreting columnar epithelial cells.

FERRET PLATE 4 FIGURES 5 & 6

STOMACH

X 80



X 600

FERRET : PLATE 5 : FIGURE 7.

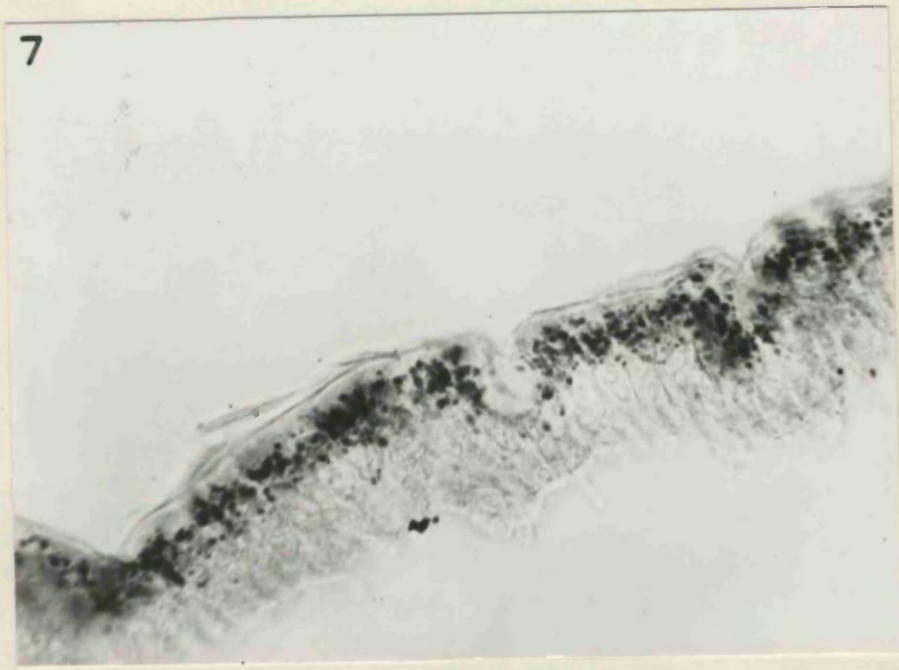
Duodenum-First Part.

Figure 7: Young Ferret Duodenum - first part
1½ hours after 'skim-cream'.
Sudan IV. (No counter-stain)
Magnification x 600.

Lining epithelium, showing fine, supranuclear, sudanophilic granules.

FERRET PLATE 5 FIGURE 7

DUODENUM Ist PART



X 600

FERRET : PLATE 6 : FIGURES 8 - 9

Intestine - Portion A.

Figure 8: Young Ferret. Fasting.
Sudan IV with Haemalum.
Magnification x 600.

Granulocytes in stroma of villus.

Granules are stained a dark, brownish-yellow colour with Sudan IV.

Figure 9: Young Ferret.
1½ hours after 'skim-cream'.
Sudan IV with Haemalum.
Magnification x 350.

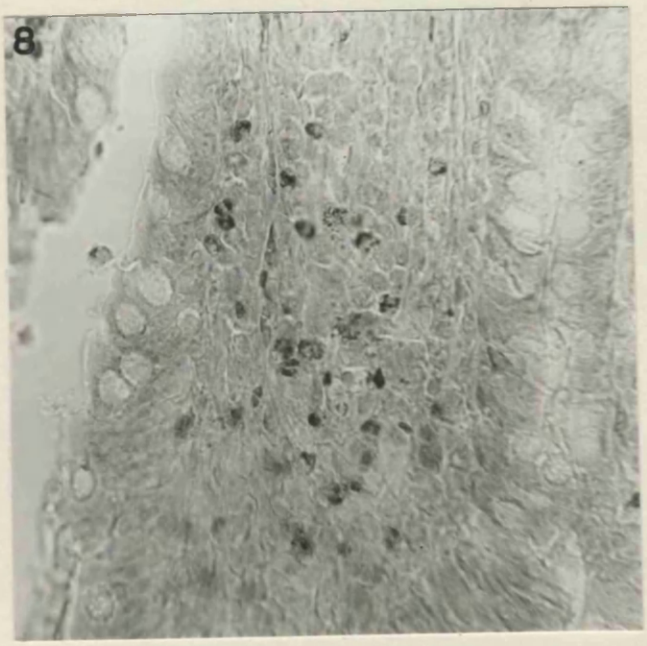
Fine, sudanophilic granules in epithelium - in supranuclear and infranuclear positions of the cells.

Granulocytes in core of villi, and in lamina propria between upper parts of crypts.

FERRET PLATE 6 FIGURES 8 & 9

INTESTINE PORTION A

8



X 600

9

X 350

FERRET : PLATE 7 : FIGURES 10 - 11

Intestine - Portion A.

Figure 10: Young Ferret.
4½ hours after 'skim-cream'.
Sudan IV.
Magnification x 600.

Some large sudanophilic droplets in epithelial cells at tips of villi.

Figure 11: Adult Ferret.
4 hours after 'skim-cream'.
Aortic Perfusion. Sudan Black with Haemalum.
Magnification x 80.

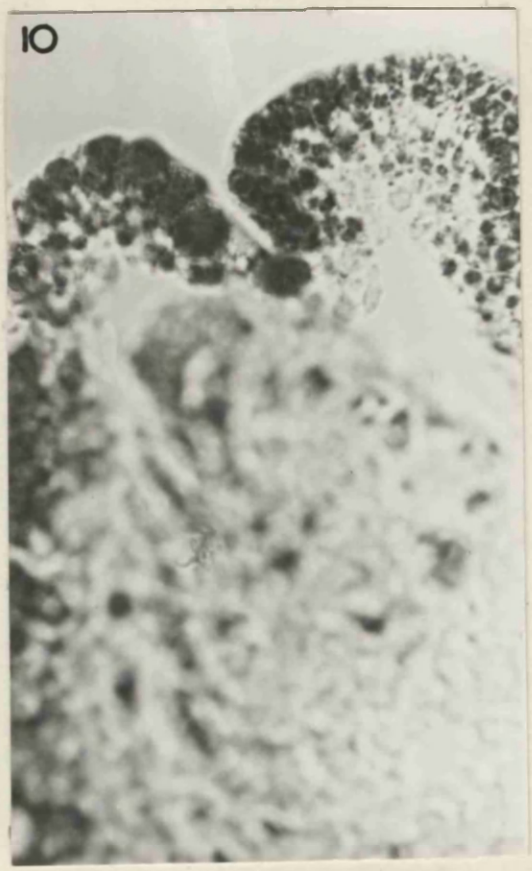
Oblique section through villi and mucosa.

Epithelium contains Sudan-stained droplets.

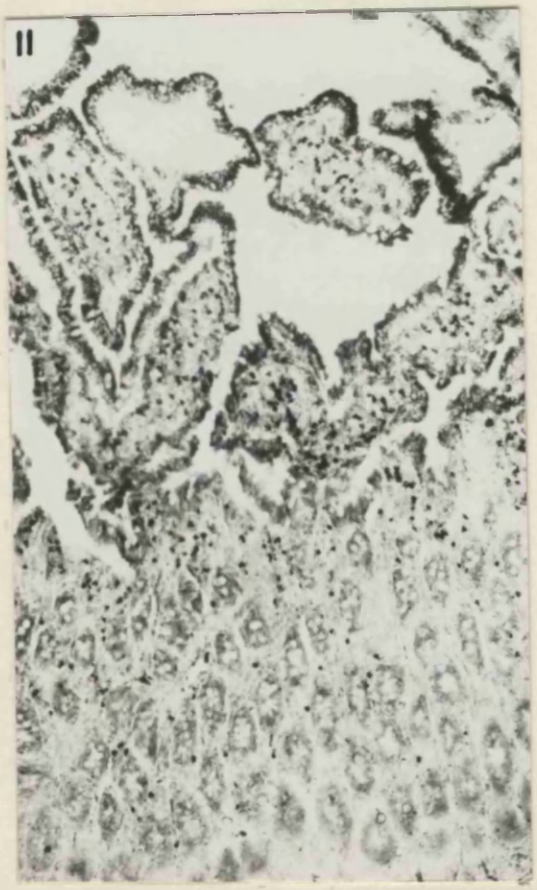
Dark-staining granulocytes crowd stroma of villi and between crypts.

FERRET PLATE 7 FIGURES 10 & 11

INTESTINE PORTION A



X 600



X 80

FERRET : PLATE 8 : FIGURES 12 - 13.

Intestine - Portions B & C.

Figure 12: Adult Ferret. Intestine - Portion B.
4 hours after 'skim-cream'.
Aortic Perfusion. Sudan Black.
Magnifications x 80.

Cross Section Intestinal wall.
Sudanophilic material in lymph vessel at junction mucosa with submucosa.

Granulocytes with Sudan-stained granules, crowd the lamina propria.

Figure 13: Adult Ferret. Intestine - Portion C.
4 hours after 'skim-cream'.
Aortic Perfusion. Sudan Black.
Magnification x 80.

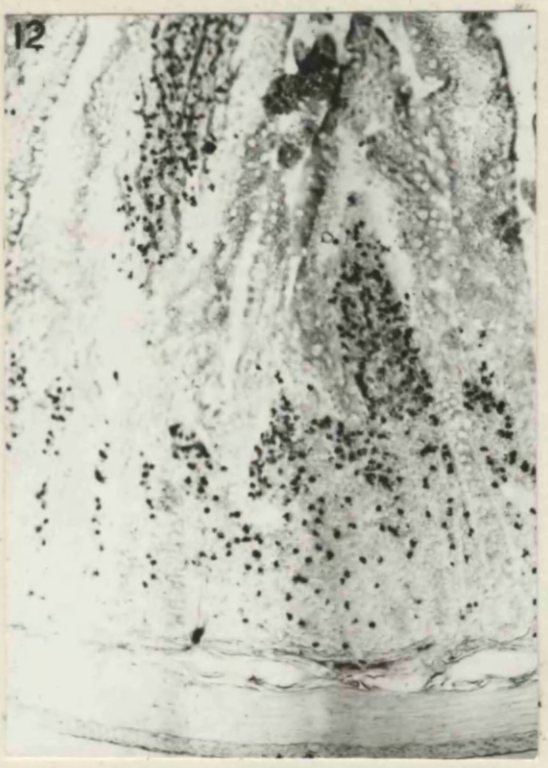
Migratory granulocytes crowd stroma of villi and between crypts.

Lymph vessels at junction mucosa and submucosa contain granular, sudanophilic material.

Villi are somewhat shorter in this portion.

FERRET PLATE 8 FIGURES 12 & 13

INTESTINE PORTION A & B



X 80

X 80

FERRET : PLATE 9 : FIGURE 14.

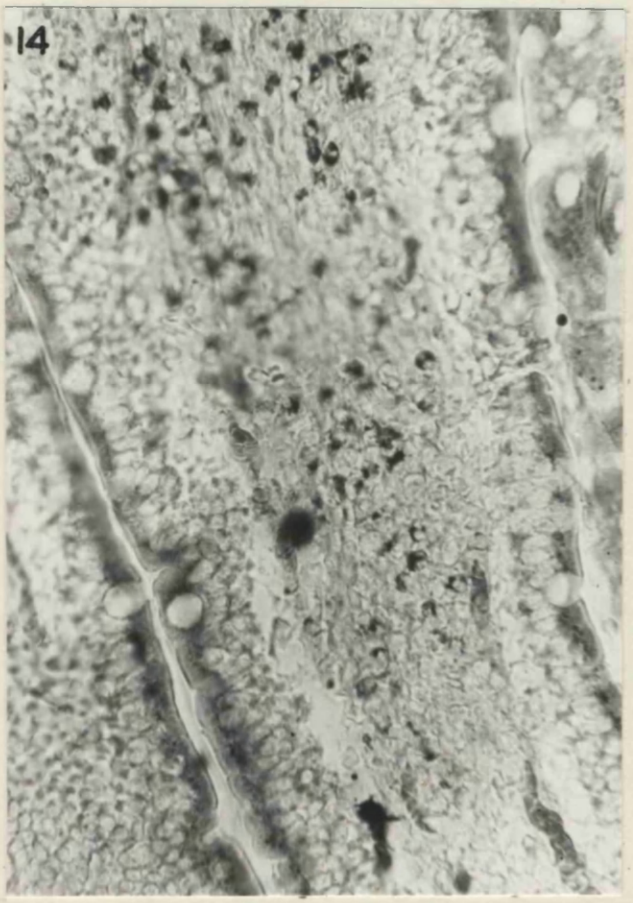
Intestine - Portion C.

Figure 14: Young Ferret.
3 hours after 'skim-cream'.
Sudan Black.
Magnification x 600.

High power view of villus. Supranuclear and infranuclear, sudanophilic granules in epithelial cells.
Central Lacteal with Sudan-stained material.
Positive-staining granulocytes in stroma of villus.

FERRET PLATE 9 FIGURE 14

INTESTINE PORTION C



X 600

FERRET : PLATE 10 : FIGURES 15 - 16.

Intestine - Portions D & E.

Figure 15: Adult Ferret. Intestine - Portion D.
4 hours after 'skim-cream'.
Aortic Perfusion.
Sudan IV with Haemalum.
Magnification x 80.

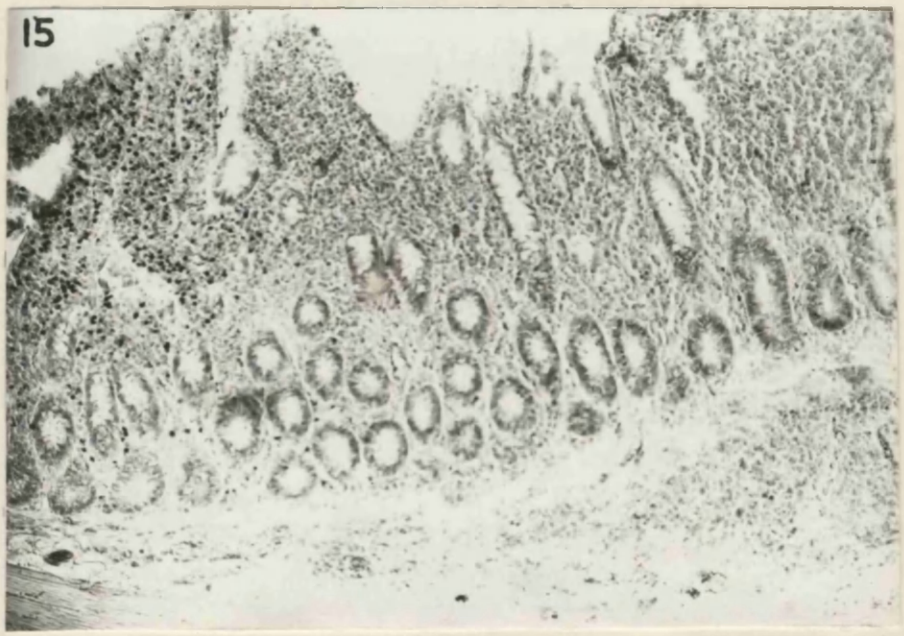
Positive-staining granulocytes in stroma of villus (on left of picture), but completely absent from dense lymphatic tissue of Peyer's patch (right ¾ of picture) and from submucosal lymphatic nodule (on extreme right of picture).

Figure 16: Adult Ferret. Intestine - Portion E.
4 hours after 'skim-cream'.
Aortic Perfusion.
Sudan Black.
Magnification x 80.

Long slender villi with positive-staining granulocytes in the lamina propria. (red blood cells in the marginal capillaries, etc., are dark in the photographic print, but are not positive with Sudan Black).

FERRET PLATE 10 FIGURES 15 & 16

INTESTINE PORTION D & E



X 80

X 80

FERRET : PLATE 11 : FIGURES 17 - 18.

Intestine - Portion F.

Figure 17: Adult Ferret. (Terminal Colon).
4 hours after 'skim-cream'.
Sudan IV with Haemalum.
Magnification x 80.

Note absence of villi. Epithelium is negative to the Sudan stain.
Scattered granulocytes in stroma between crypts.
(Red blood cells in b.vs. are darkened in photograph).

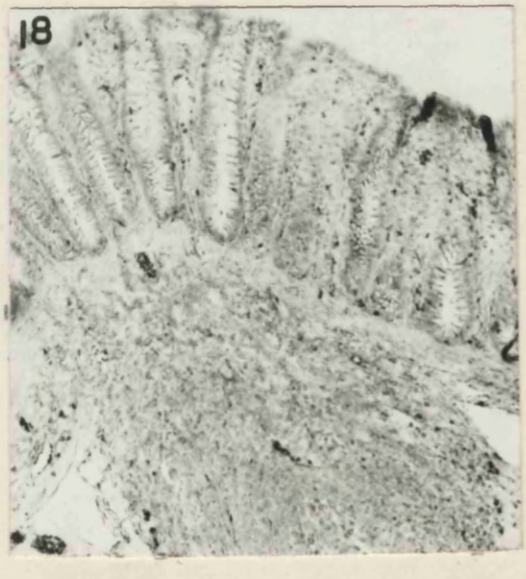
Figure 18: Adult Ferret. (Terminal Colon).
4 hours after 'skim-cream.'
Sudan IV with Haemalum.
Magnification x 80.

Note absence of villi and of positive-Sudan-staining in epithelium.
Occasional migratory granulocytes in stroma between crypts.
Solitary lymphatic nodule in submucosa is negative to the Sudan-stain.
(Red blood cells are dark in the photographic print).

FERRET PLATE II FIGURES 17 & 18

INTESTINE PORTION F

X 80



X 80

FERRET : PLATE 12 : FIGURES 19 - 21.

Intestine - Portion F.

Figure 19: Young Ferret.
Fasting.
Sudan Black with Haemalum.
Magnification x 600.

Note absence of villi. High power view of surface epithelium. Sudanophilic granules of varying sizes, in surface epithelial cells, chiefly in supranuclear position, but also, in some cells, very fine granules deep to nucleus.

Figure 20: Young Ferret.
1½ hours after 'skim-cream'.
Sudan Black.
Magnification x 600.

Similar, supranuclear, sudanophilic granules in cells of epithelium.

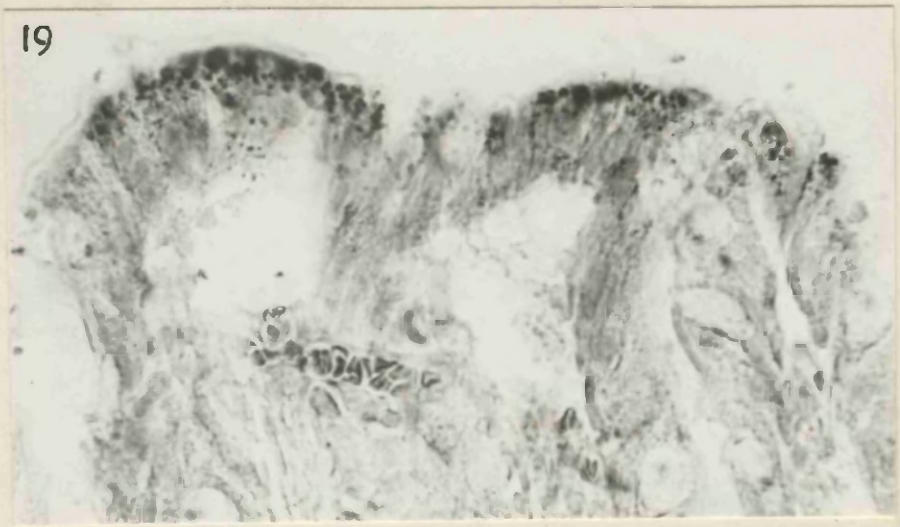
Figure 21: Young Ferret.
4½ hours after 'skim-cream'.
Sudan IV with Haemalum.
Magnification x 600.

Great increase in size and number of the strongly sudanophilic droplet inclusions in surface epithelium.

FERRET PLATE 12 FIGURES 19 - 21

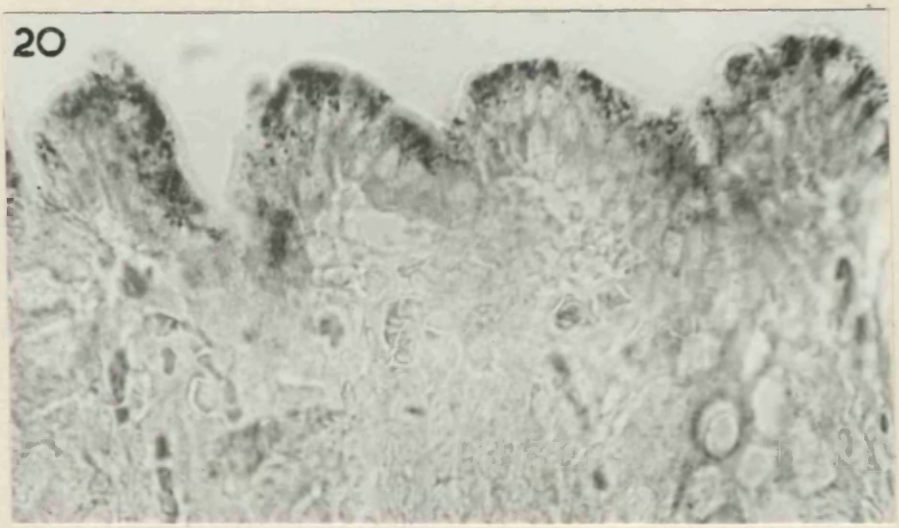
INTESTINE PORTION F

19



X 600

20



X 600

21

X 600

FERRET : PLATE 13 : FIGURES 22 - 23.

Mesenteric Lymph Node.

Figure 22: Adult Ferret.
4 hours after 'skim-cream'.
Aortic Perfusion.
Sudan IV.
Magnification x 150.

Strongly Sudan-stained macrophages in medulla.
Pale Sudan-stained lymph in all loose lymphatic tissue.
Some granular staining in fixed R.E. cells of the germinal centres.

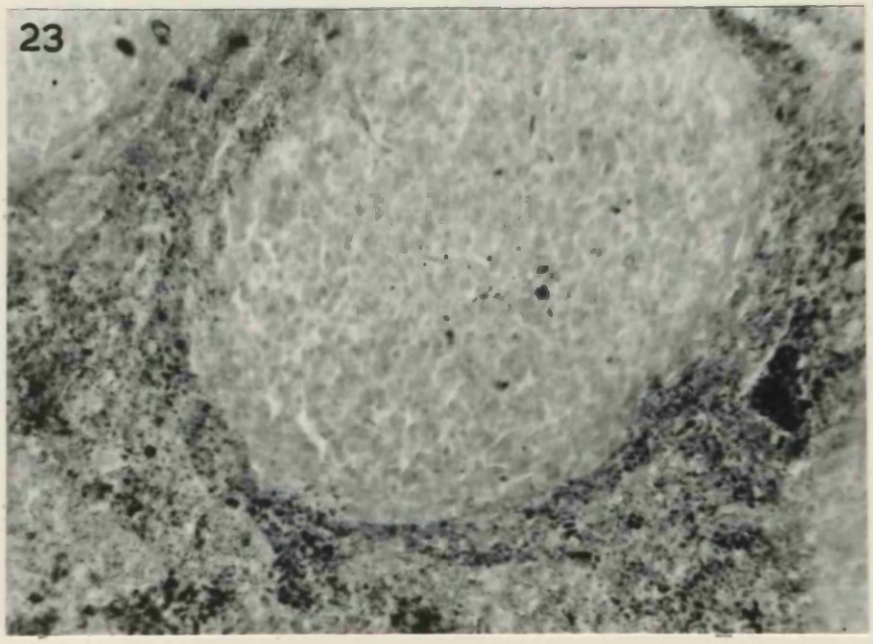
Figure 23: Adult Ferret.
4 hours after 'skim-cream'.
Aortic Perfusion.
Sudan IV with Haemalum.
Magnification x 150.

As for Figure 22.

FERRET PLATE 13 FIGURES 22 & 23

MESENTERIC LYMPH NODE

X 150



X 150

FERRET : PLATE 14 : FIGURE 24.

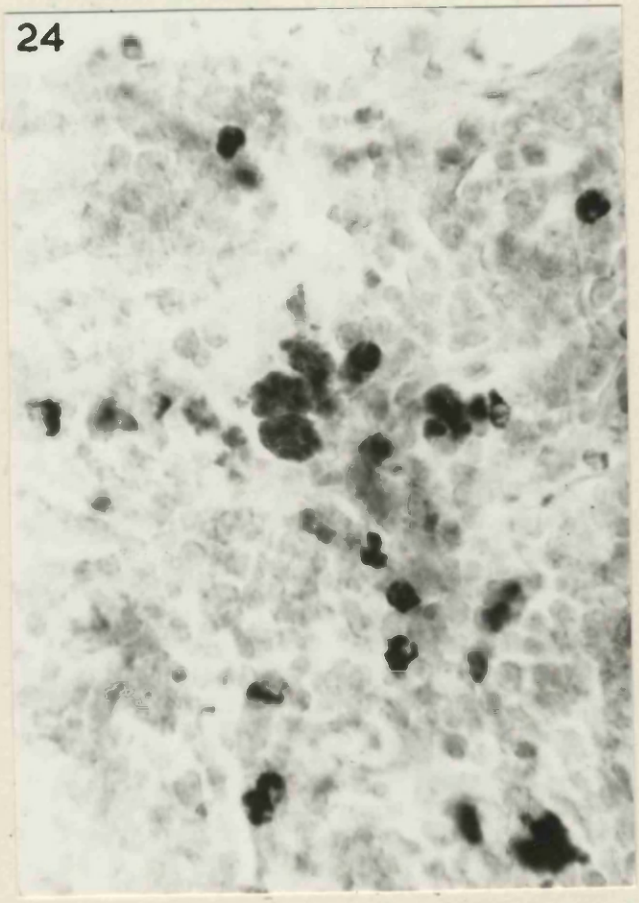
Mesenteric Lymph Node.

Figure 24: Adult Ferret.
4 hours after 'skim-cream'.
Ordinary Fixation.
Sudan Black with Haemalum.
Magnification x 600.

Macrophages, containing Sudan-stained globules, in loose lymphatic (sinus) tissue. (A few smaller granulocytes are also seen).

FERRET PLATE 14 FIGURE 24

MESENTERIC LYMPH NODE



X 600

FERRET : PLATE 15 : FIGURES 25- 26.

Pyloro-duodenal Lymph Node.

Figure 25: Young Ferret (Lymph Node from wall of first part of the duodenum).
1½ hours after 'skim-cream'.
Sudan Black with Haemalum.
Magnification x 80.

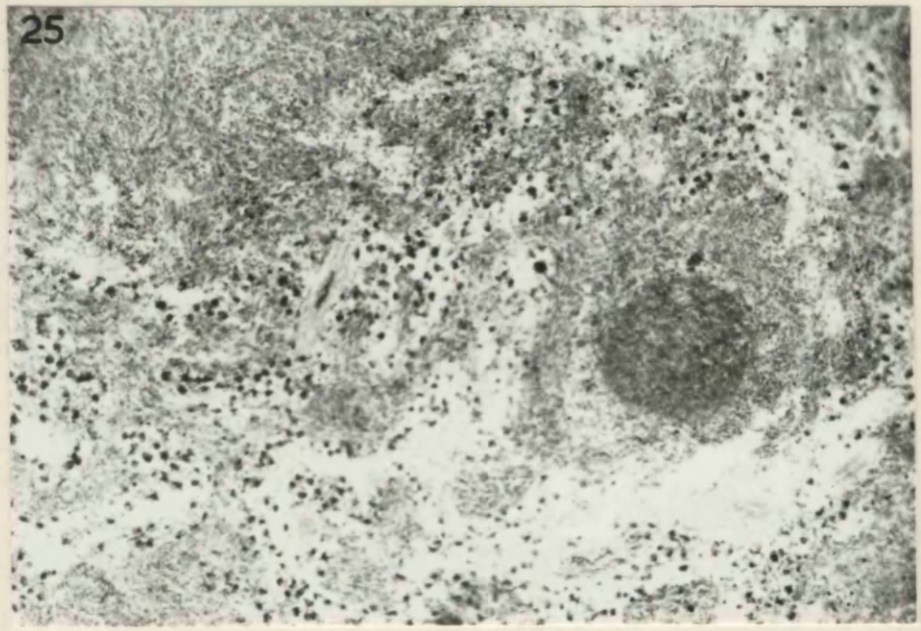
Positive-staining macrophages, of the Reticulo-endothelial type, in the loose lymphatic tissue.

Figure 26: Young Ferret. (Lymph Node from wall of the first part of the duodenum).
4½ hours after 'skim-cream'.
Sudan IV with Haemalum.
Magnification x 80.

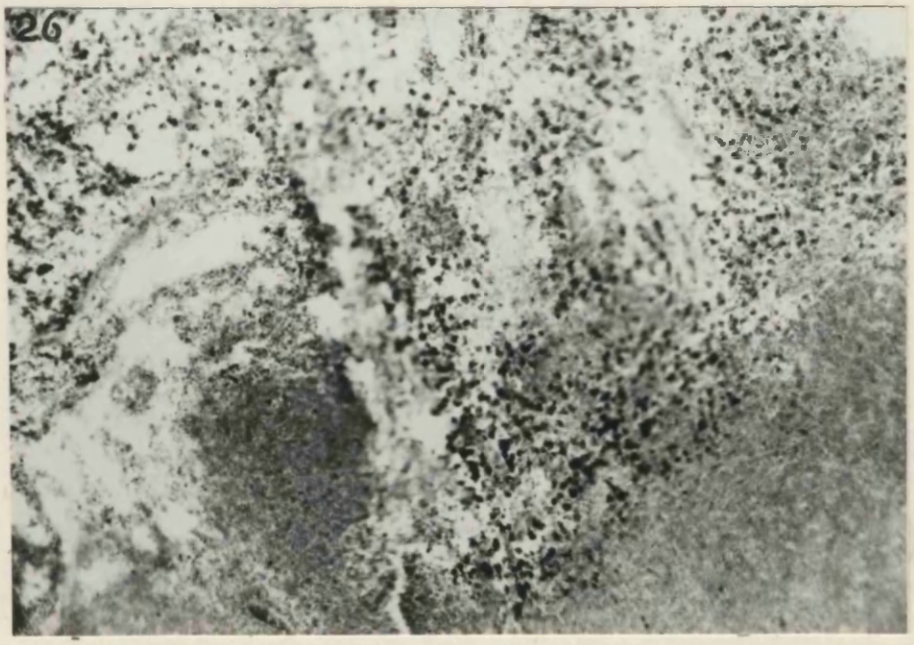
Increased numbers of macrophages in loose lymphatic tissue.

FERRET PLATE 15 FIGURES 25 & 26

PYLORO DUODENAL LYMPH NODE



X 80



X 80

FERRET : PLATE 16 : FIGURES 27 - 28.

Lymph Node from Intestine - Portion E.

Figure 27: Young Ferret.
Fasting.
Sudan Black with Haemalum.
Magnification x 80.

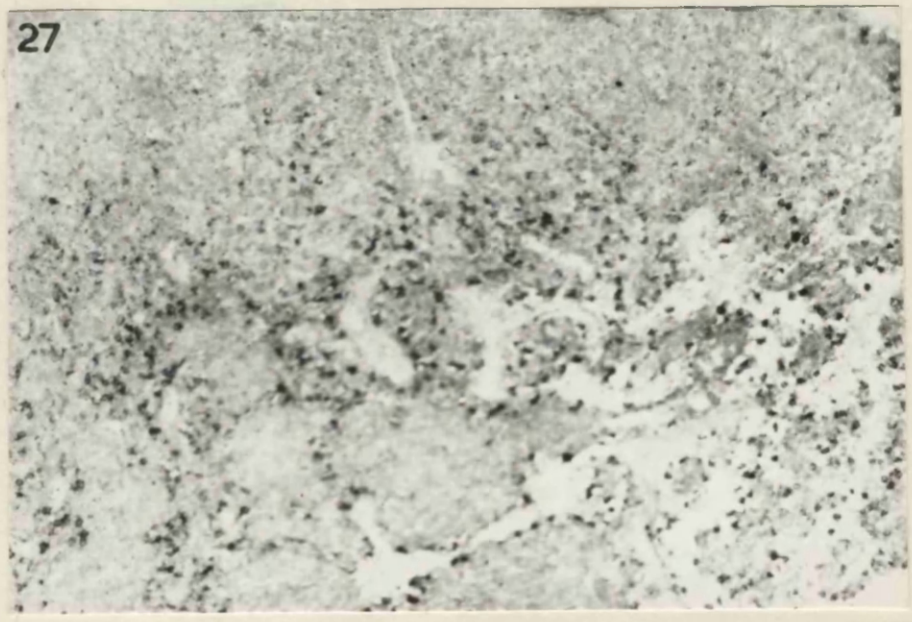
Positive-staining macrophages in loose lymphatic tissue.

Figure 28: Young Ferret.
4½ hours after 'skim-cream'.
Sudan IV.
Magnification x 80.

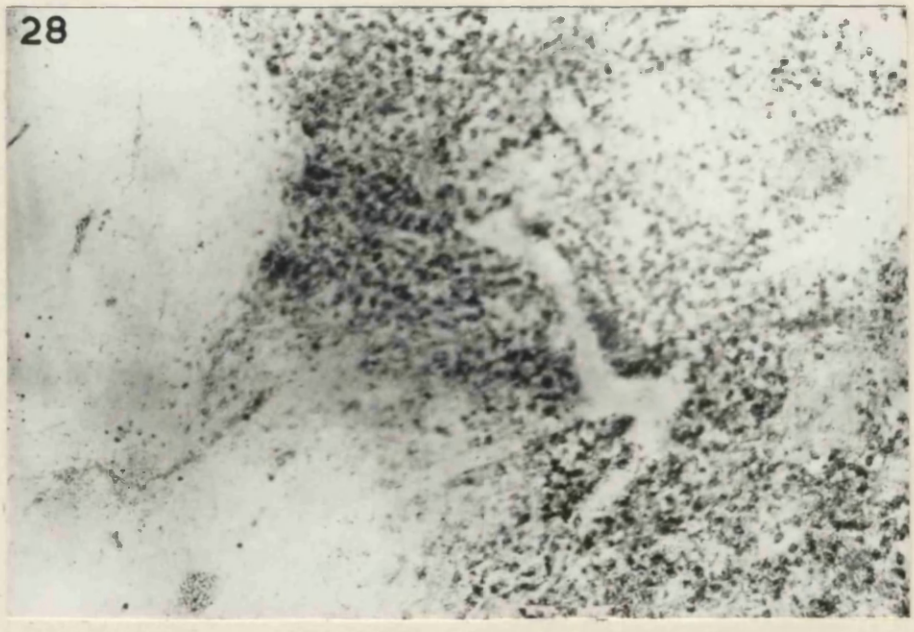
Increased numbers of macrophages in loose lymphatic tissue.

FERRET PLATE 16 FIGURES 27 & 28

LYMPH NODE FROM INTESTINE PORTION E



X 80



X 80

MOUSE

PLATES 1 - 15.

(FIGURES 1 - 31).

MOUSE : PLATE 1 : FIGURES 1 - 2

Forestomach.

Figure 1: Fasting.
Sudan IV with Haemalum.
Magnification x 500.

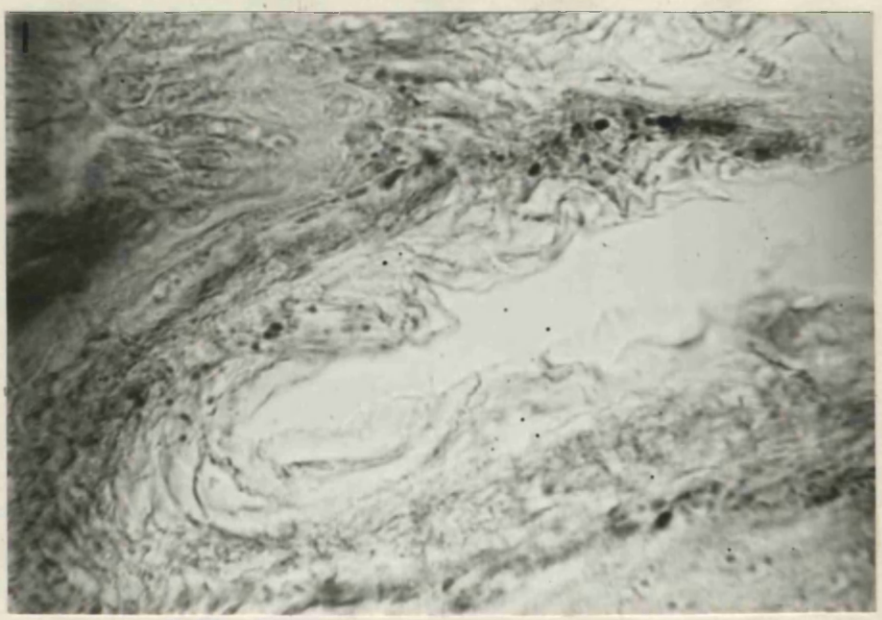
Stratified Squamous Epithelium of keratinised type.
Discrete, sudanophilic droplets in upper layers of stratum corneum.
(Stratum Spinosum on left of picture).

Figure 2: 12 hours after 'skim-cream'.
Sudan Black with Haemalum.
Magnification x 250.

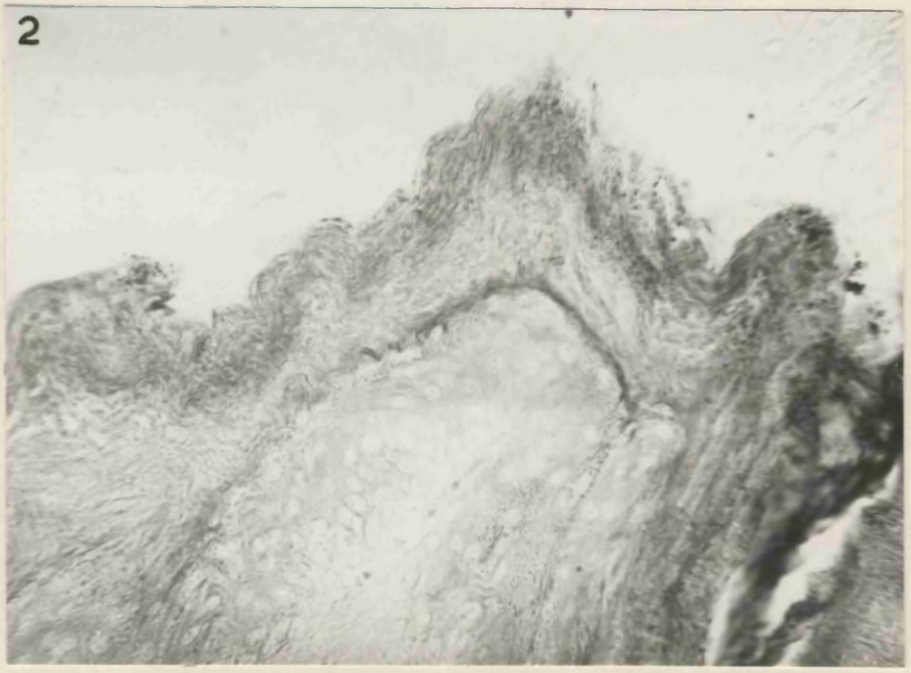
Cornified layers show diffuse Sudan colouration, with strongly sudanophilic surface layers.
(Stratum granulosum is prominently basophilic in picture).

MOUSE PLATE 1 FIGURES 1 & 2

FORESTOMACH



X 500



X 250

MOUSE : PLATE 2 : FIGURES 3 - 5

Forestomach.

Figure 3: 1½ hours after 'skim-cream'.
Sudan IV with Haemalum.
Magnification x 150.

Diffuse Sudan-staining throughout the cornified layers, with more densely stained material adhering to surface.

Figure 4: 1½ hours after 'skim-cream.'
Sudan Black.
Magnification x 250.

Dense staining in surface cornified layers.

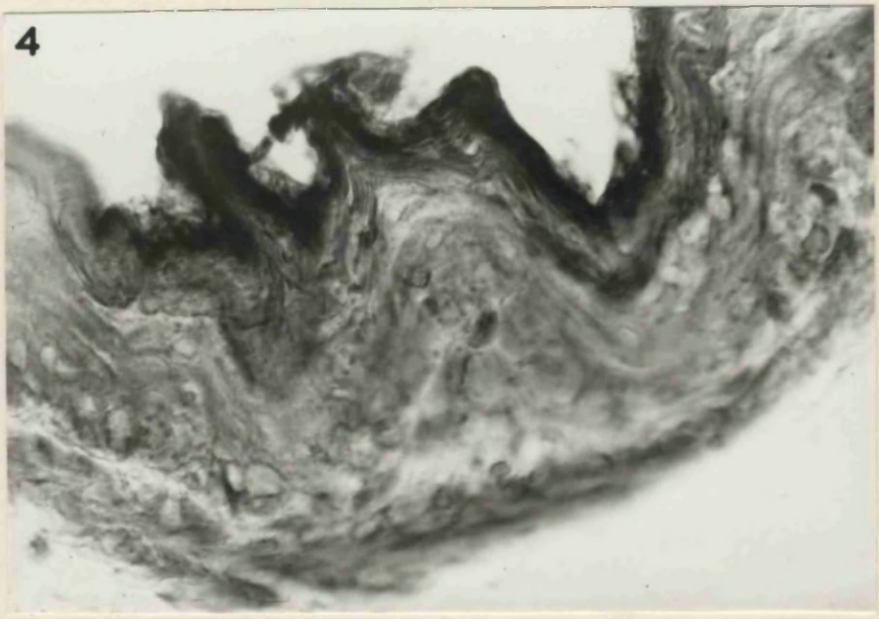
Figure 5: 6 hours after 'skim-cream'.
Sudan Black.
Magnification x 500.

Stratum Corneum only. (Upper surface (a) on left of picture).
Pale, diffuse Sudan-colouring throughout, with strong Sudan-staining in surface layers.

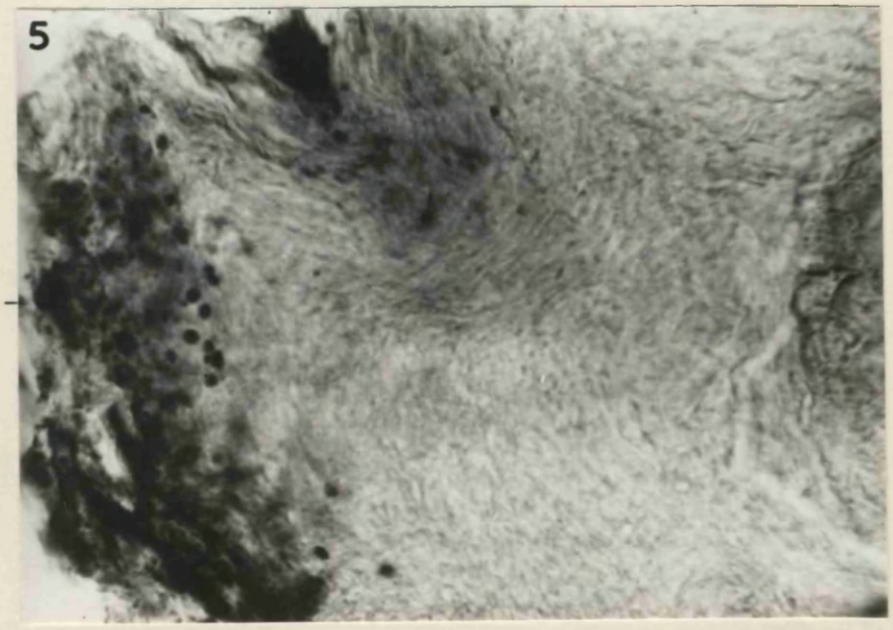
MOUSE PLATE 2 FIGURES 3 – 5

FORESTOMACH

X 150



X 250



X 500

MOUSE : PLATE 3 : FIGURES 6 - 7

Forestomach.

Figure 6: 3 hours after 'skim-cream'.
Sudan IV with Haemalum.
Magnification x 500.

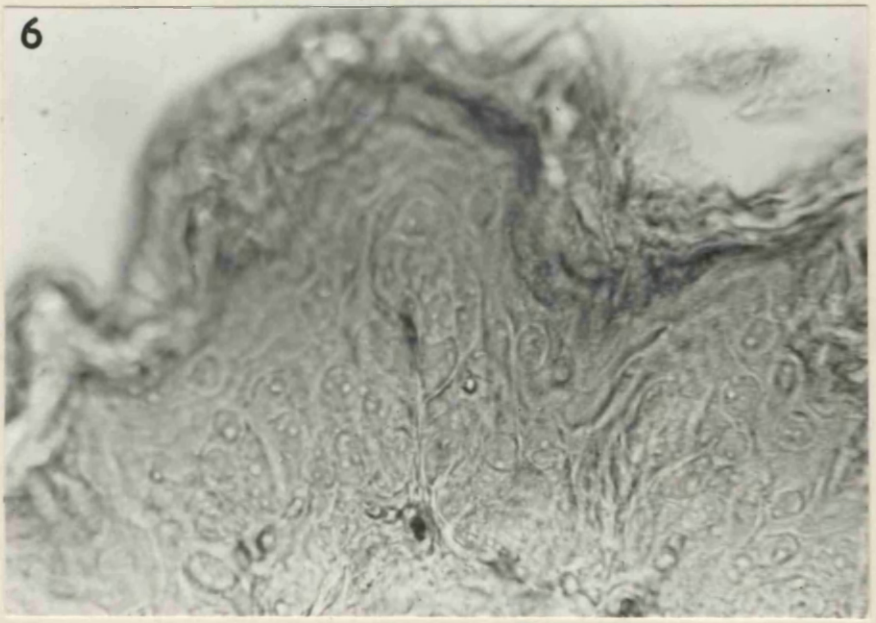
Sudan-stained granulocytes in lamina propria deep to basement membrane of surface epithelium.
Pale Sudan-tinting throughout corneum.

Figure 7: 4½ hours after 'skim-cream'.
Sudan IV with Haemalum.
Magnification x 500.

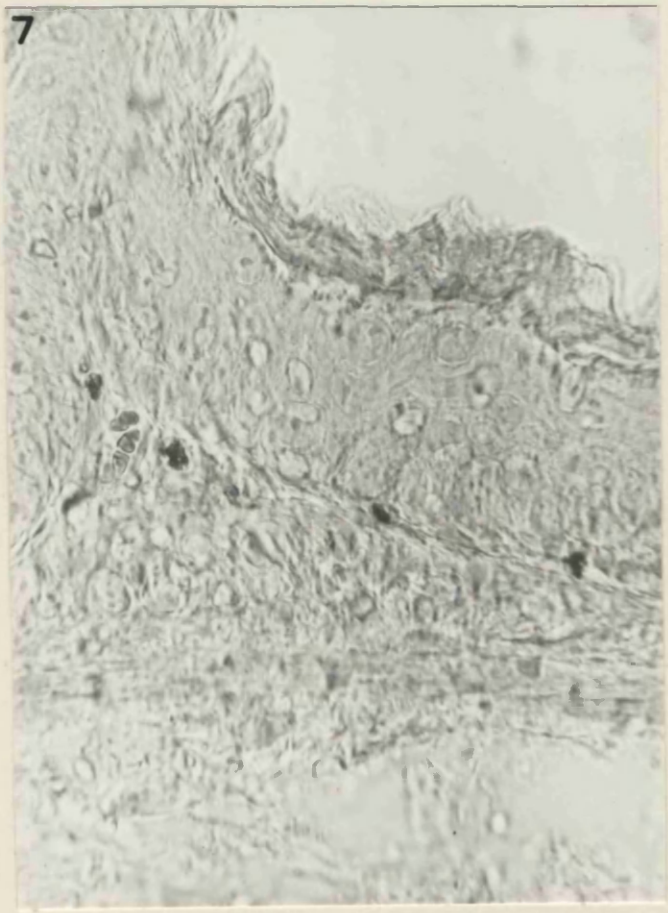
Granulocytes in lamina propria deep to basement membrane.
Pale Sudan-tinting of cornified layers.

MOUSE PLATE 3 FIGURES 6 & 7

FORESTOMACH



X 500



X 500

MOUSE : PLATE 4 : FIGURE 8.

Body of Stomach.

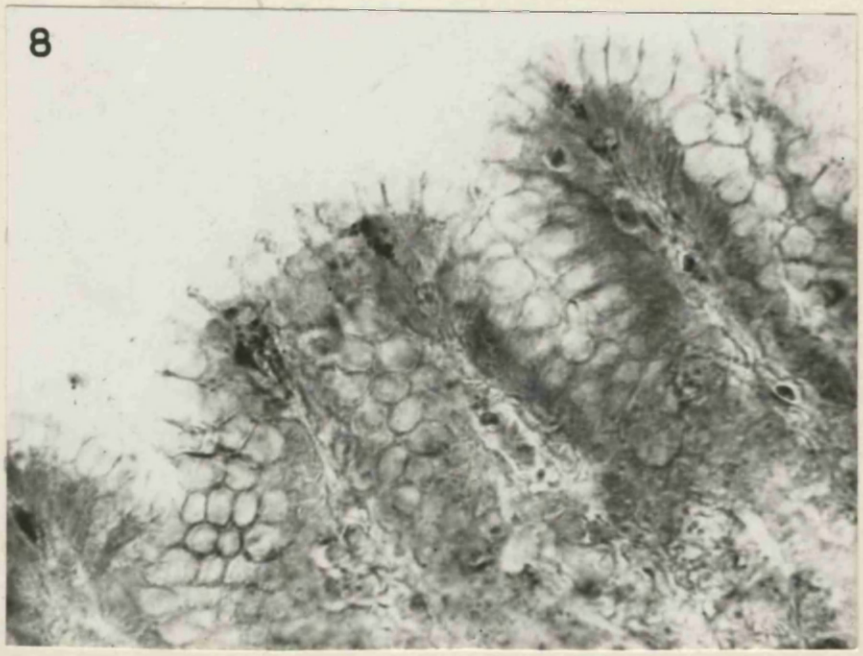
Figure 8: 3 hours after 'skim-cream'.
Sudan IV with Haemalum.
Magnification x 600.

Mucus-secreting, columnar epithelium.

Fine, infranuclear, sudanophilic granules in some surface cells.

MOUSE PLATE 4 FIGURE 8

BODY OF STOMACH



X 600

MOUSE : PLATE 5 : FIGURES 9 - 10.

Duodenum - First Part.

Figure 9: 3 hours after 'skim-cream'.
Sudan Black.
Magnification x 150.

Very fine infranuclear and supranuclear, sudanophilic granulation in epithelial cells.

Occasional migratory granulocytes between crypts and at root of villi.

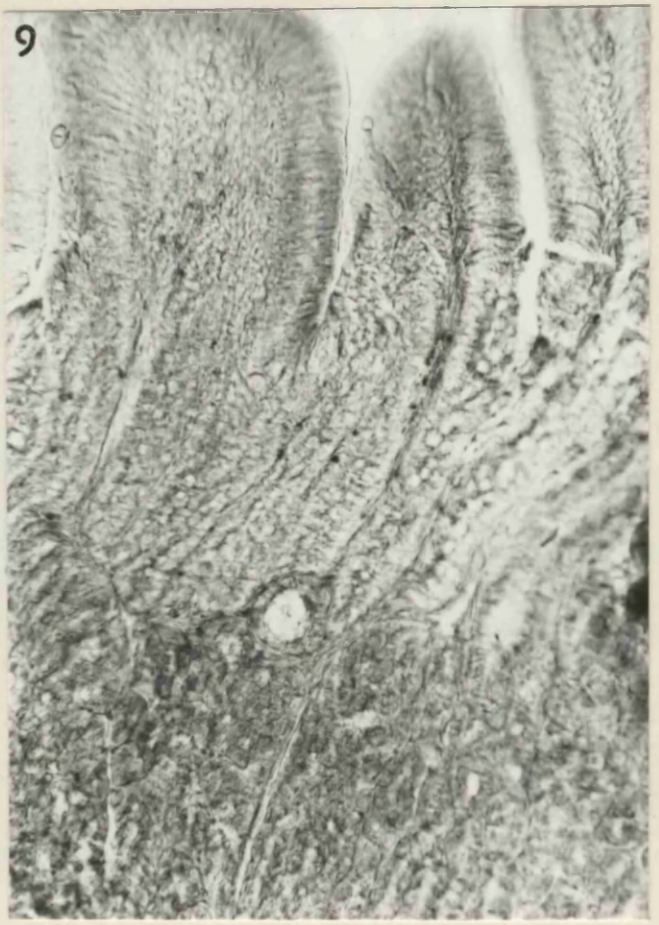
Figure 10: 3 hours after 'skim-cream'.
Sudan IV with Haemalum.
Magnification x 600.

Fine infranuclear, sudanophilic droplets in columnar epithelium covering villi.

MOUSE PLATE 5 FIGURES 9 & 10

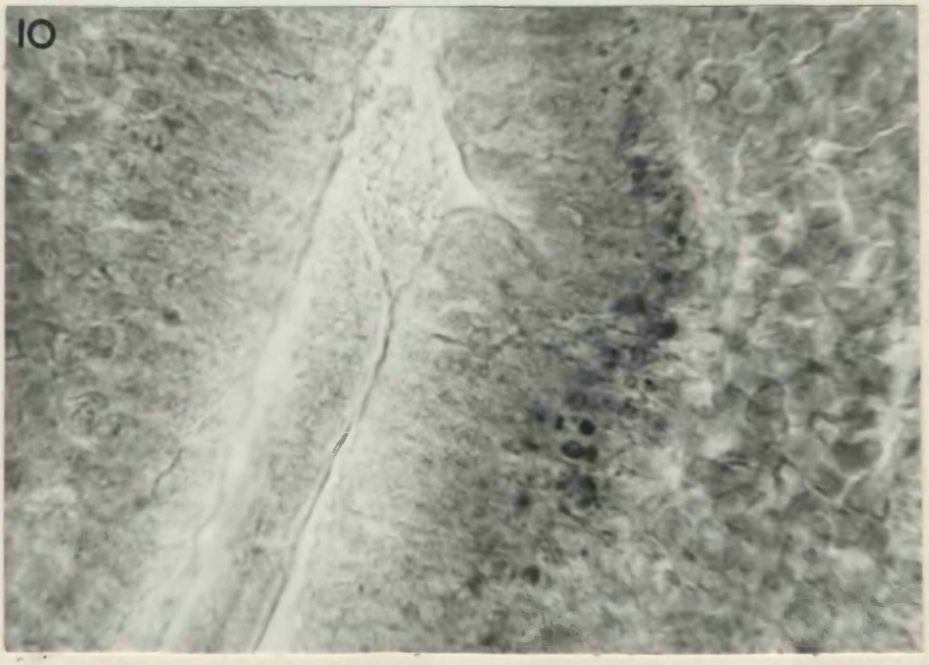
DUODENUM 1st PART

9



X 150

10



X 600

MOUSE : PLATE 6 : FIGURES 11 - 12.

Duodenum - First Part.

Figure 11: 3 hours after 'skim-cream'.
Sudan Black with Haemalum.
Magnification x 600.

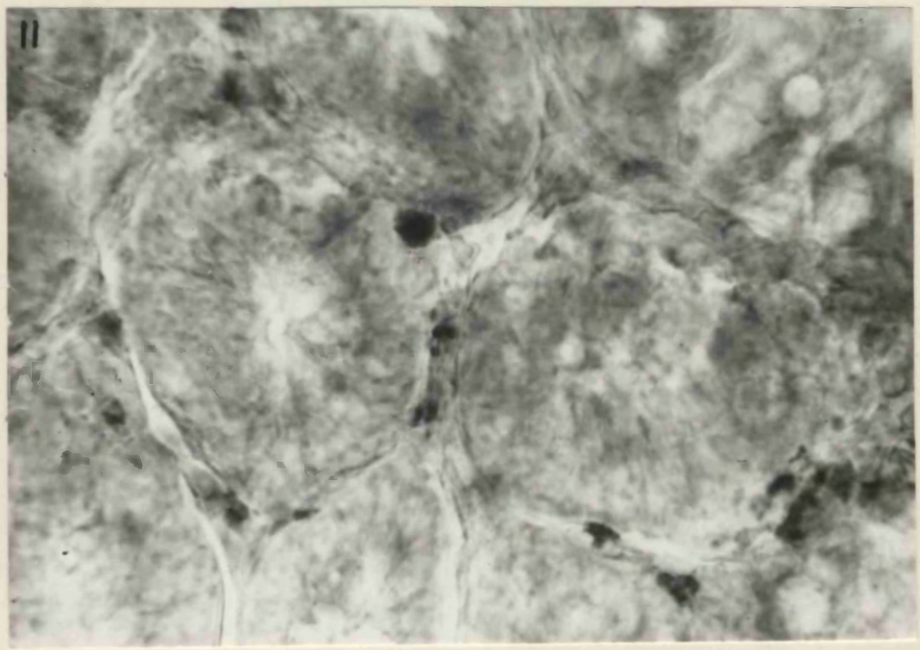
Cross-section of crypts of Lieberkuhn.
Sudan-stained granulocytes in lamina propria between crypts.

Figure 12: 6 hours after 'skim-cream'.
Sudan Black.
Magnification x 125.

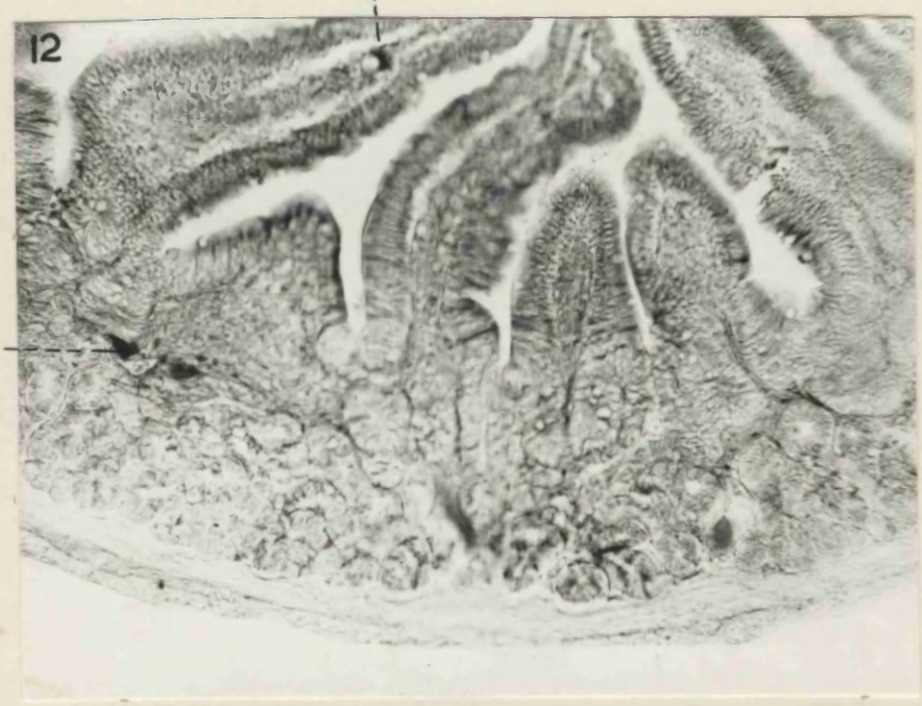
Villi with submucosa and smooth muscle layers; showing sudanophilic material in several submucosal lymphatics (a) and in one central lacteal (b).
(Occasional granulocytes are just visible in core of villi and between crypts.)

MOUSE PLATE 6 FIGURES 11 & 12

DUODENUM 1st PART



X 600



X125

MOUSE : PLATE 7 : FIGURES 13 - 14.

Jejunum - Upper Third.

Figure 13: 1½ hours after 'skim-cream'.
Sudan Black.
Magnification x 150.

Low power view of villi, etc., showing extensive involvement of epithelium with Sudan-stained globules.

One central lacteal is visible containing sudanophilic material.

(Granulocytes are seen between crypts in lower third of mucosa).

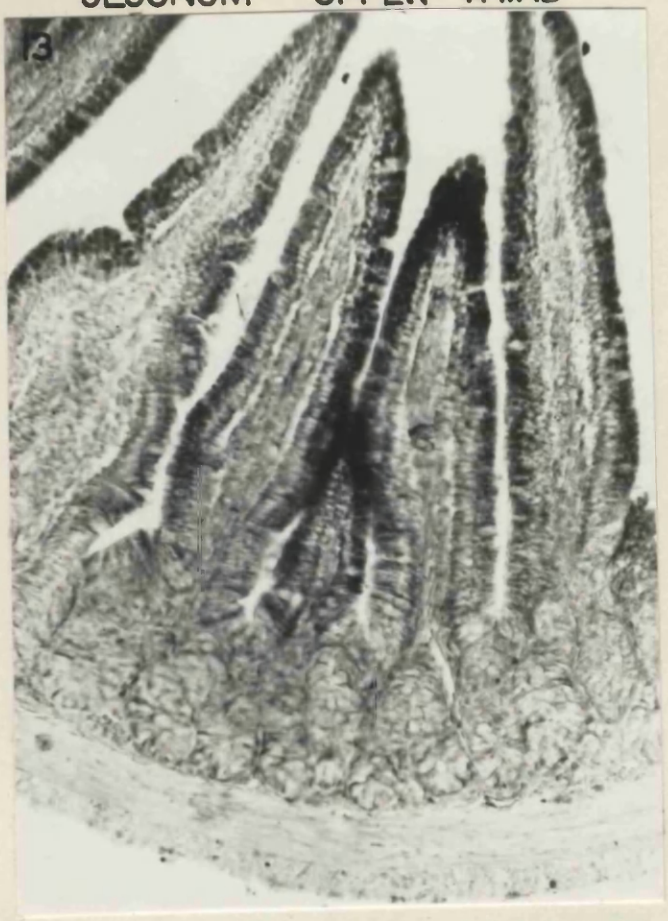
Figure 14: 1½ hours after 'skim-cream'.
Sudan IV with Haemalum.
Magnification x 600.

Epithelium with small, infranuclear granules and larger, supranuclear droplets of sudanophilic material.

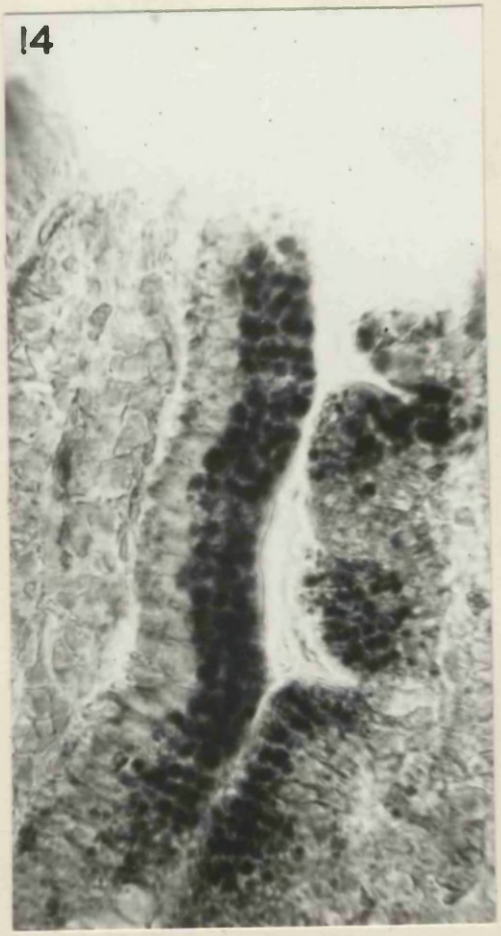
Some lie free in the stroma of villus.

MOUSE PLATE 7 FIGURES 13 & 14

JEJUNUM UPPER THIRD



X 150



X 600

MOUSE : PLATE 8 : FIGURES 15 - 17.

Jejunum - Upper Third.

Figure 15: 3 hours after 'skim-cream'.
Sudan Black.
Magnification x 350.

Tip of villus, partly in surface view, showing grading in droplet size from tip to base of villus. (? one droplet in process of absorption, showing 'hour-glass' constriction as it passes through the striated border of the cell).

Figure 16: 4½ hours after 'skim-cream'.
Sudan IV with Haemalum.
Magnification x 600.

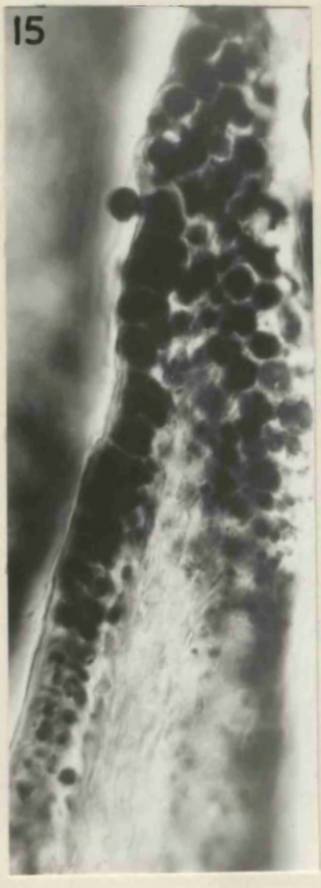
High power view villus, showing grading of droplet size from above downwards in villus, and from without inwards in epithelial cells.

Figure 17: 4½ hours after 'skim-cream'.
Sudan IV with Haemalum.
Magnification x 150.

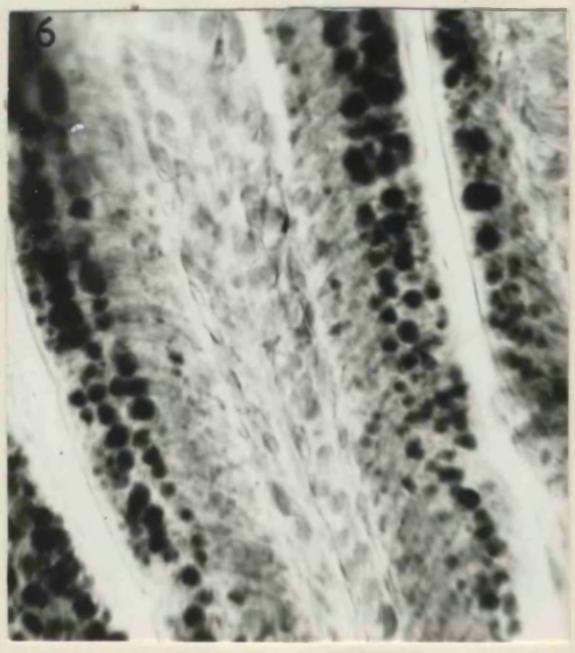
Low power view of villi. Grading of droplet size seen. Free droplets in stroma. (One villus in surface view).

MOUSE PLATE 8 FIGURES 15 – 17

JEJUNUM UPPER THIRD



X 350



X 6

X 150

MOUSE : PLATE 9 : FIGURES 18 - 20.

Jejunum - Upper Third.

Figure 18: 6 hours after 'skim-cream'.
Sudan Black.
Magnification x 150.

Mucosa and submucosa.
Sudan-stained granulocytes in lamina propria between crypts.

Figure 19: 6 hours after 'skim-cream'.
Sudan Black.
Magnification x 600.

Supranuclear, sudanophilic droplets in some epithelial cells.

Figure 20: 6 hours after 'skim-cream'.
Sudan Black.
Magnification x 600.

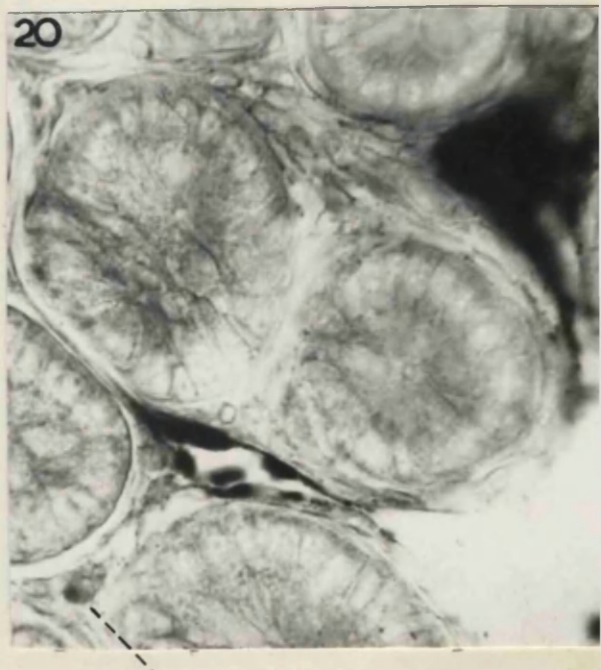
Cross-section crypts of Lieberkuhn, showing positive-staining lymphatics in lamina propria.
One granulocyte in lower left of picture. *(a)*

MOUSE PLATE 9 FIGURES 18-20

JEJUNUM UPPER THIRD

X 150

X 600



X 600 (a)

MOUSE : PLATE 10 : FIGURES 21 - 22.

Jejunum - Upper Third.

Figure 21: 3 hours after 'skim-cream'.
Sudan Black with Haemalum.
Magnification x 600.

Sudan-stained granulocytes (a) between crypts in mucosa.

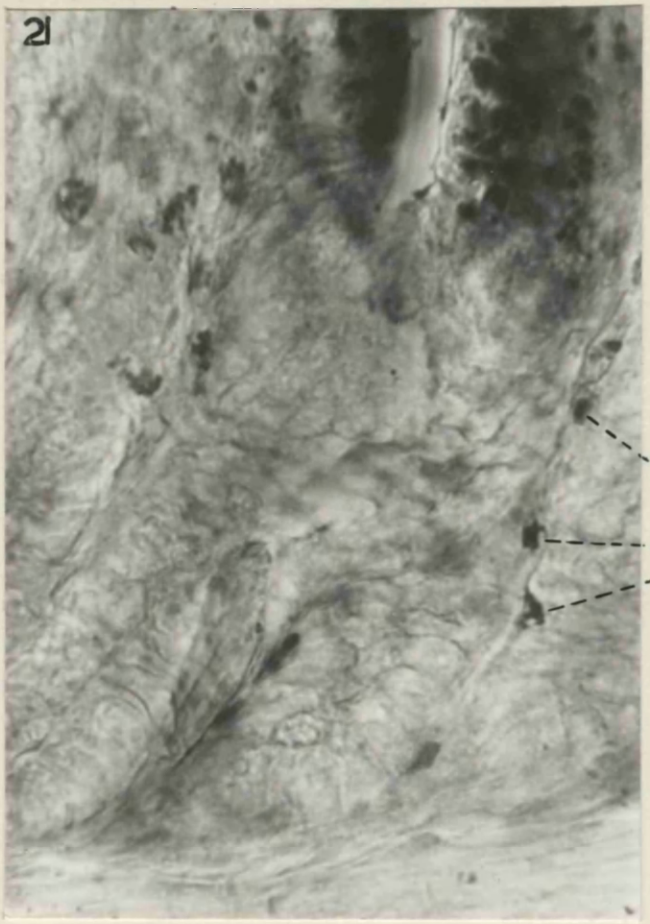
Figure 22: 12 hours after 'skim-cream'.
Sudan Black.
Magnification x 150.

Low power view of mucosa.
Residual, supranuclear, sudanophilic droplets present in some cells of the epithelium.
Two central lacteals are seen in villi, and one between crypts in lower third of mucosa.
Occasional granulocytes are noted in stroma.

MOUSE PLATE 10 FIGURES 21 & 22

JEJUNUM UPPER THIRD

21



X 600

22

X 150

MOUSE : PLATE 11 : FIGURES 23 - 24.

Jejunum - Lower Third.

Figure 23: 1½ hours after 'skim-cream'.
Sudan Black with Haemalum.
Magnification x 600.

Minute, supranuclear and infranuclear, sudanophilic droplets in epithelium.

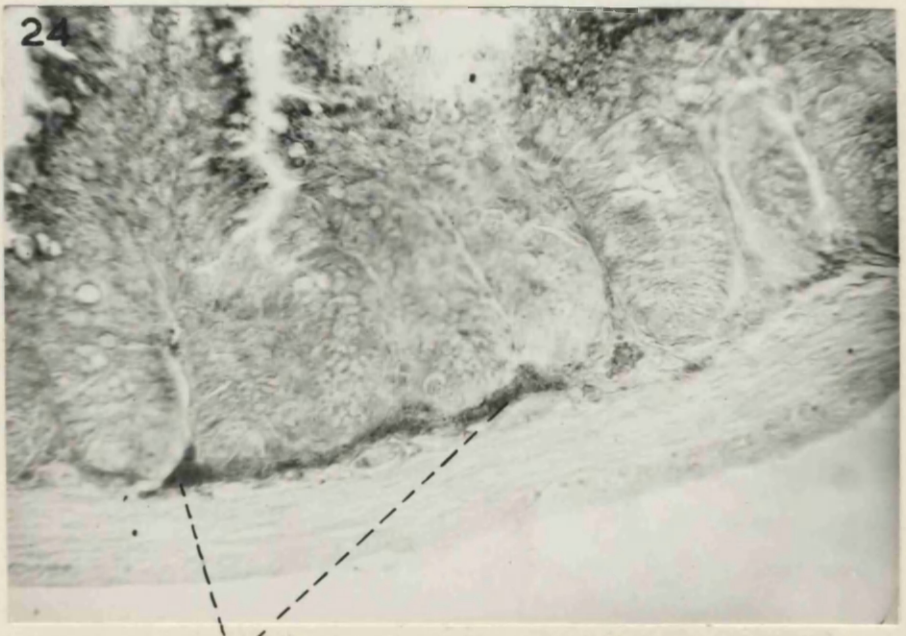
Figure 24: 6 hours after 'skim-cream'.
Sudan IV with Haemalum.
Magnification x 250.

Sudanophilic material in submucosal lymph vessel (*d*) (cut in longitudinal section).

MOUSE PLATE 11 FIGURES 23 & 24

JEJUNUM LOWER THIRD

X 600



X 250

MOUSE : PLATE 12 : FIGURES 25 - 26.

Caecum.

Figure 25: Fasting.
Sudan Black.
Magnification x 600.

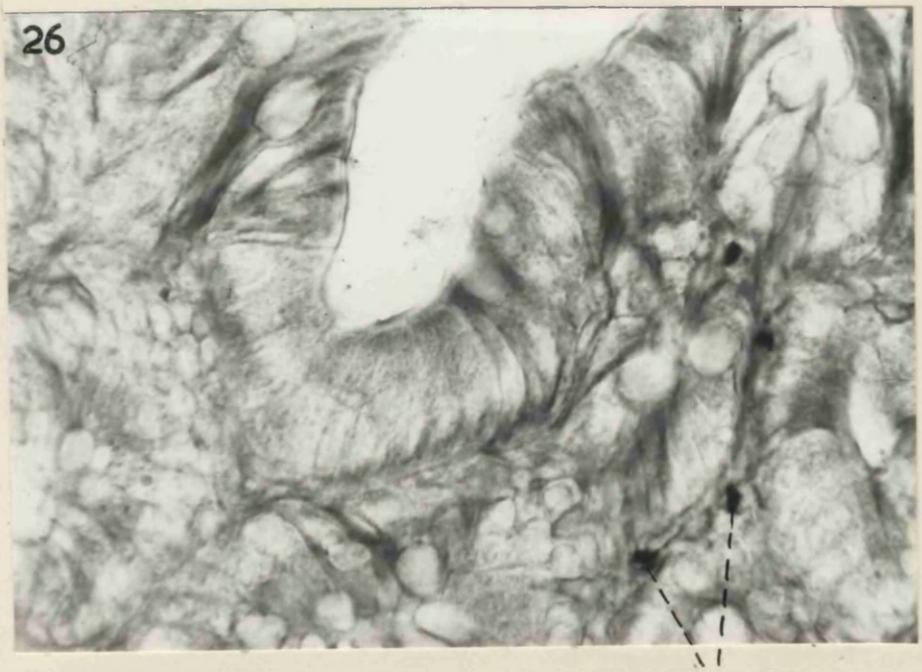
Note absence of villi.
Infranuclear, sudanophilic, 'dust-like' particles in lining epithelial cells.

Figure 26: 1½ hours after 'skim-cream'.
Sudan Black.
Magnification x 600.

Epithelium negative.
Granulocytes in lamina propria between crypts near base of mucosa (a).

CAECUM

X 600



X 600

MOUSE : PLATE 13 : FIGURES 27 - 28.

Caecum.

Figure 27: 4½ hours after 'skim-cream'.
Sudan Black.
Magnification x 80.

Mucosa and submucosa, showing distribution of sudanophilic droplets in epithelial cells (predominantly in infranuclear position).

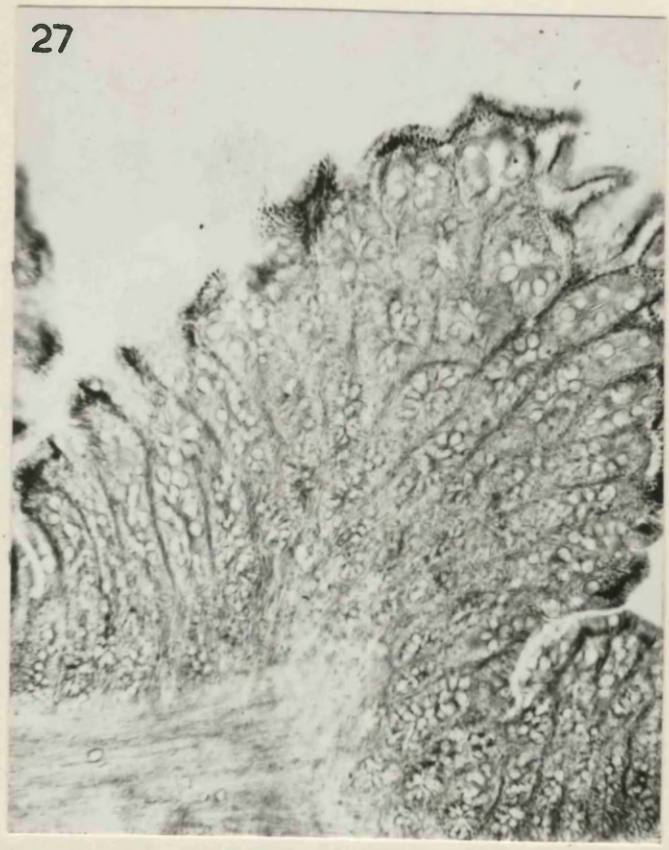
Figure 28: 4½ hours after 'skim-cream'.
Sudan Black.
Magnification x 600.

High power view of epithelium showing fine, infranuclear, sudanophilic particles.

MOUSE PLATE 13 FIGURES 27 & 28

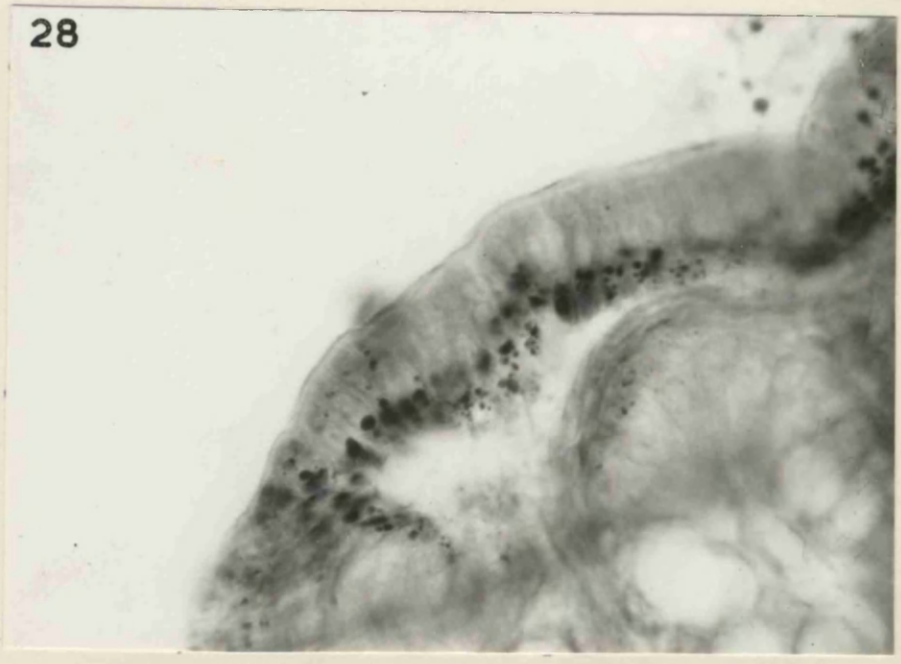
CAECUM

27



X 80

28



X 600

MOUSE : PLATE 14 : FIGURES 29 - 30.

Proximal Colon.

Figure 29: Fasting.
Sudan Black.
Magnification x 150.

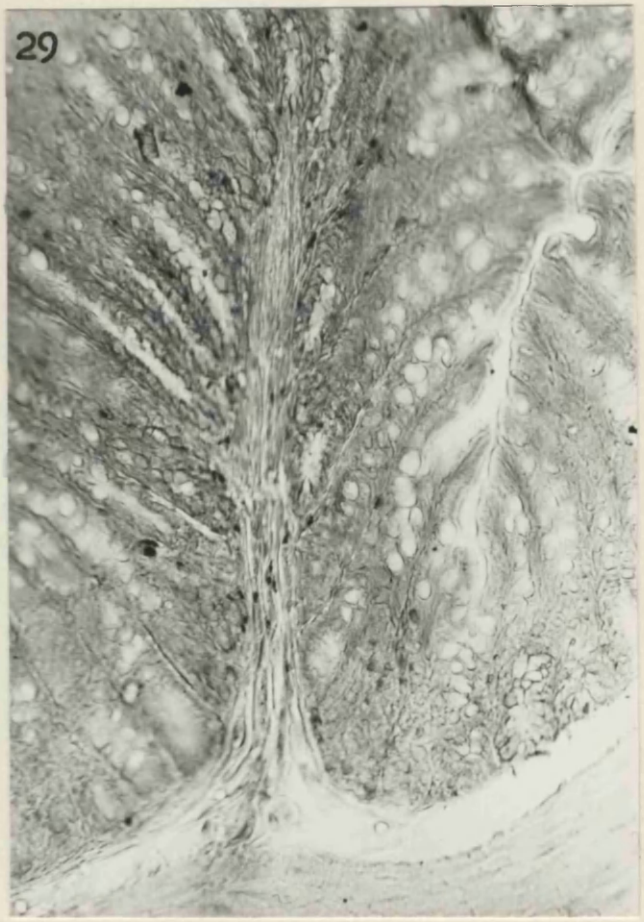
Sudan-stained granulocytes in lamina propria between crypts and in submucosa, especially where projected into a mucosal fold.

Figure 30: 4½ hours after 'skim-cream'.
Sudan Black.
Magnification x 600.

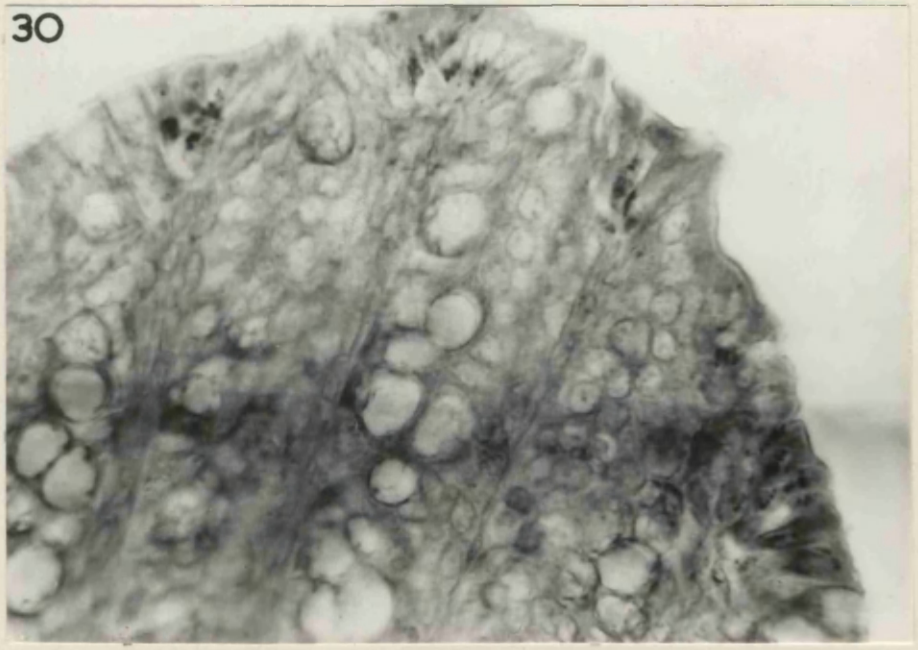
Fine, dust-like, sudanophilic granules (especially in infranuclear position) in some lining epithelial cells.

MOUSE PLATE 14 FIGURES 29 & 30

PROXIMAL COLON



X 150



X 600

MOUSE ::PLATE 15 : FIGURE 31.

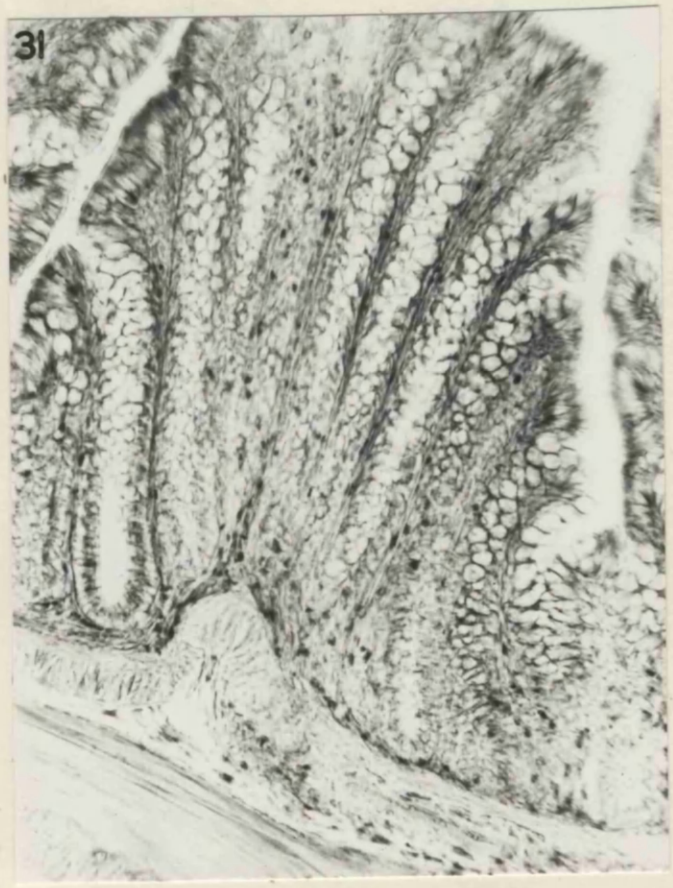
Distal Colon.

Figure 31: 3 hours after 'skim-cream'.
Sudan Black.
Magnification x 150.

Sudanophilic granulocytes in lamina propria and in submucosa.

MOUSE PLATE 15 FIGURE 31

DISTAL COLON



X 150

HAMSTER.

PLATE 1.

FIGURES 1 & 2.

HAMSTER : PLATE 1 : FIGURES 1 & 2.

Figure 1: Caecum. Fasting.
Sudan IV with Haemalum.
Magnification x 350.

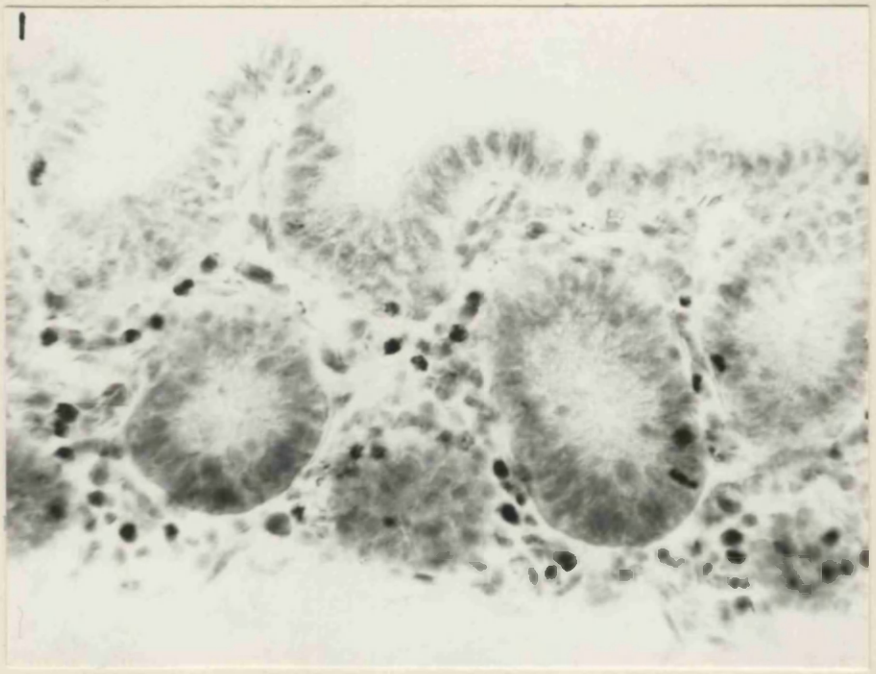
Mucosa in oblique section.
Migratory granulocytes with Sudan-stained granules, in lamina propria - several in migration through epithelium in lower parts of crypts.

Figure 2: Proximal Colon. 6 hours after 'skim-cream'.
Sudan IV with Haemalum.
Magnification x 150.

Migratory granulocytes (granules stained with Sudan) migrating through lamina propria and surface epithelium.
Pale diffuse cytoplasmic colouring with Sudan in luminal aspect of surface epithelium.

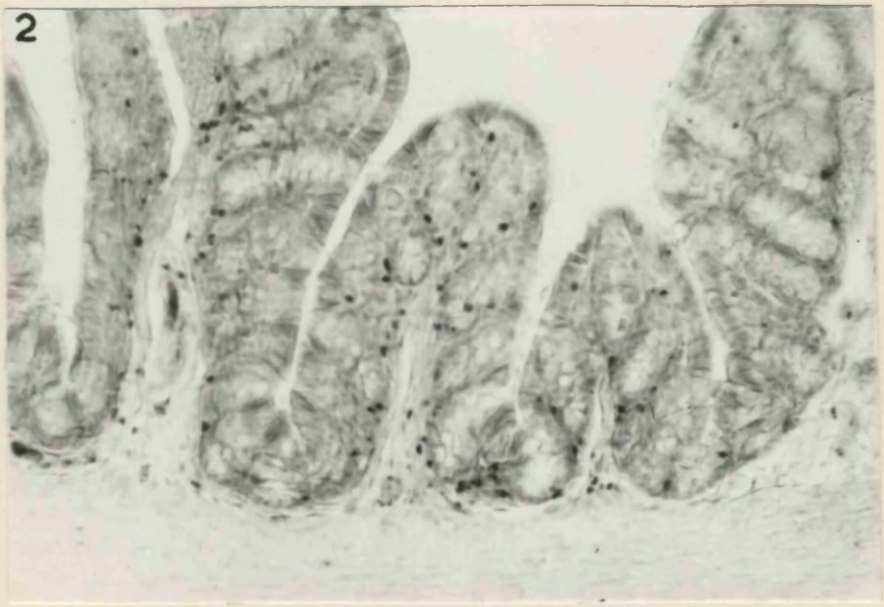
HAMSTER PLATE 1 FIGURES 1 & 2

CAECUM



X 350

PROXIMAL COLON



X 150

RAT.

PLATES 1 - 3.

(FIGURES 1 - 4)

RAT : PLATE 1 : FIGURES 1 - 2.

Figure 1: Forestomach.
3½ hours after 'skim-cream'.
Sudan Black with Haemalum.
Magnification x 150.

Dense Sudan-staining in stratum corneum.

Granulocytes with densely stained granules in lamina propria; several within submucosal blood vessels (a).

Figure 2: Forestomach.
Fasting.
Sudan IV with Haemalum.
Magnification x 250.

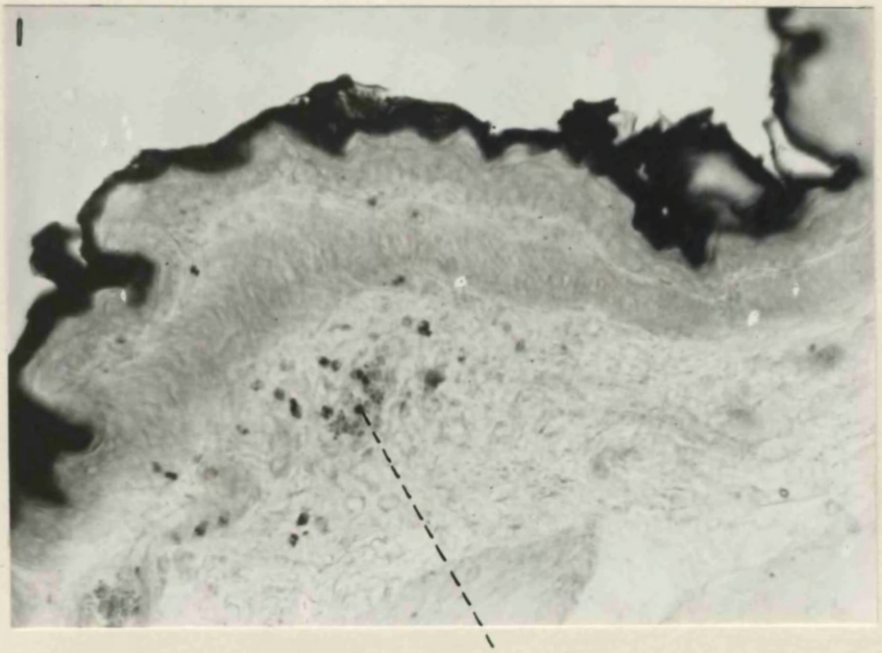
Prominent basophilic Stratum Granulosum.

Stratum Corneum virtually unstained with Sudan IV.

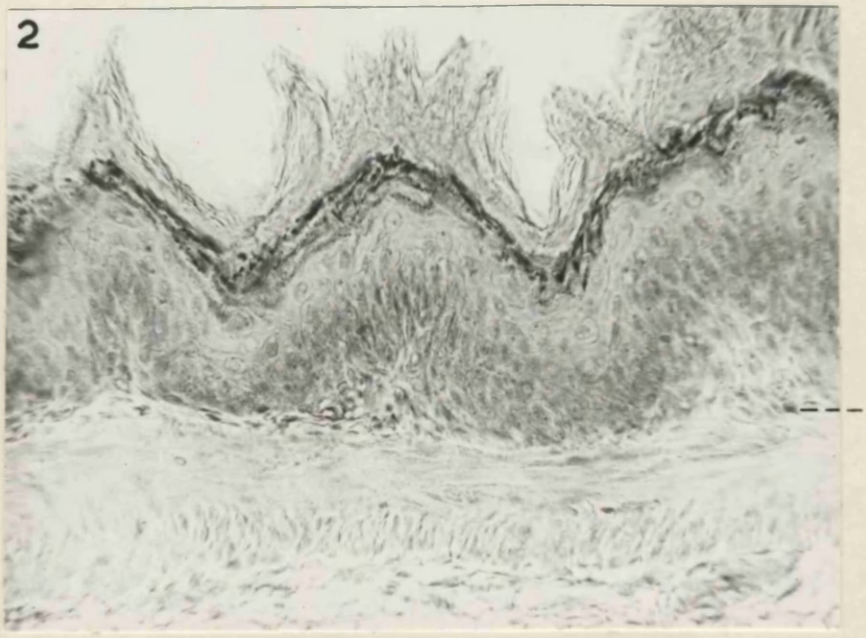
Occasional granulocytes deep to basement membrane.(a).

RAT PLATE 1 FIGURES 1 & 2

FORESTOMACH



X 150



X 250

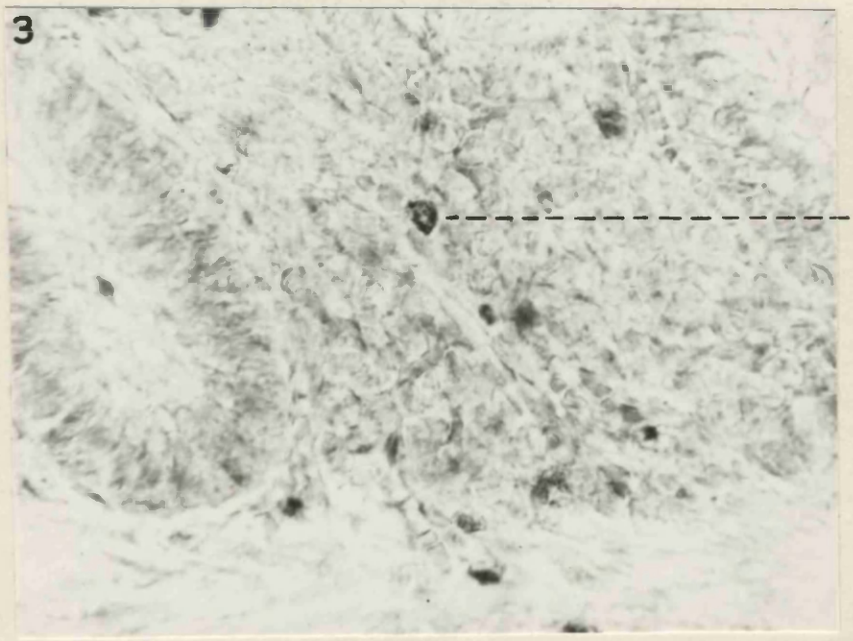
RAT : PLATE 2 : FIGURE 3.

Figure 3: Jejunum. 1½ hours after 'skim-cream'.
Sudan IV with Haemalum.
Magnification x 500.

Granulocytes near base of mucosa
and between crypts (a).

RAT PLATE 2 FIGURE 3

JEJUNUM



X 500

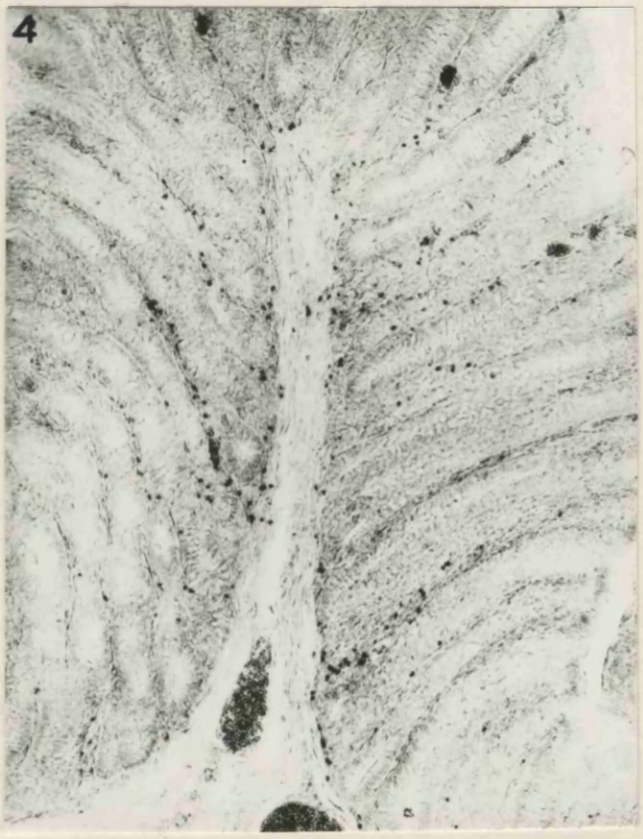
RAT : PLATE 3 : FIGURE 4.

Figure 4: Proximal Colon.
1½ hours after 'skim-cream'.
Sudan Black.
Magnification x 80.

Invasion of mucosal stroma by granulocytes.
(red blood cells in submucosal and mucosal blood vessels are dark in photographic print).

RAT PLATE 3 FIGURE 4

PROXIMAL COLON



X 80

GUINEA-PIG

PLATES 1 - 6

(FIGURES 1 - 10)

Guinea-Pig : Plate 1 : Figures 1 - 2

Figure 1: Duodenum - First Part.

Fasting.

Sudan IV with Haemalum.
Magnification x 150.

Basophilic epithelial cells in base of crypt epithelium.

Figure 2: Duodenum - First Part.

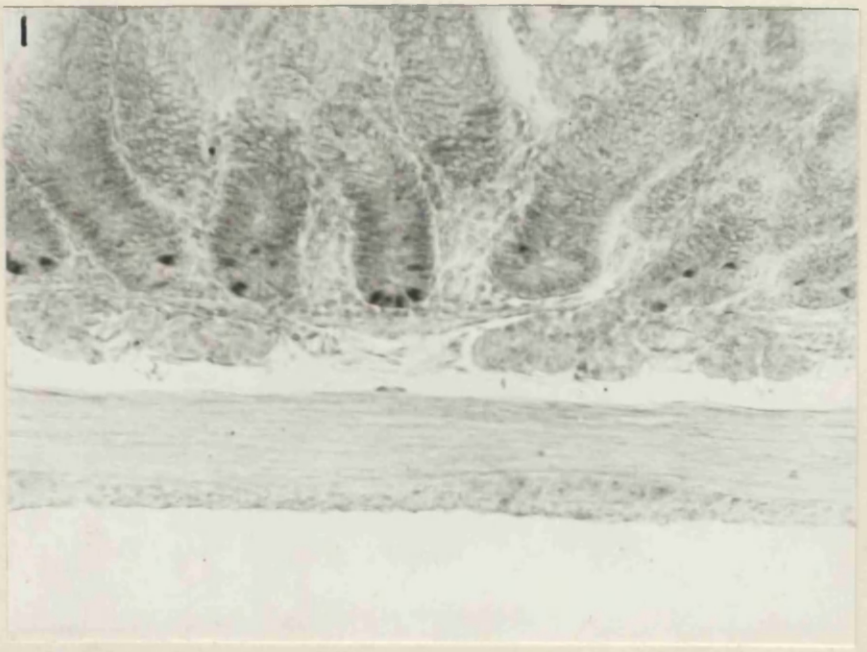
Fasting.

Sudan IV with Haemalum.
Magnification x 600.

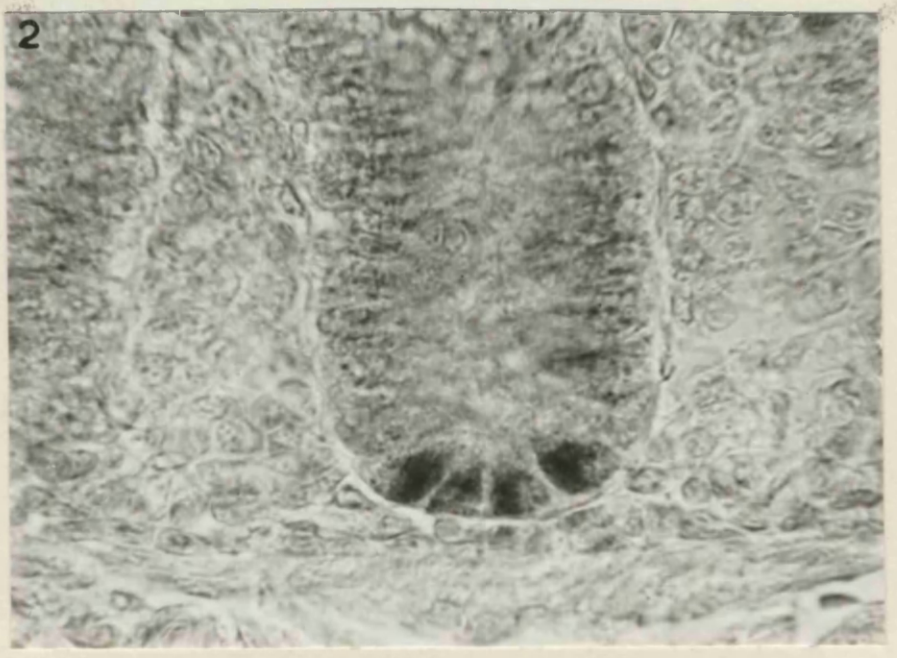
High power view of base of crypt - supranuclear, basophilic granules in epithelial cells at base of crypt.

GUINEA PIG PLATE I FIGURES 1 & 2

DUODENUM 1st PART



X 150



X 600

Guinea-Pig : Plate 2 ; Figure 3.

Figure 3: Upper Jejunum.

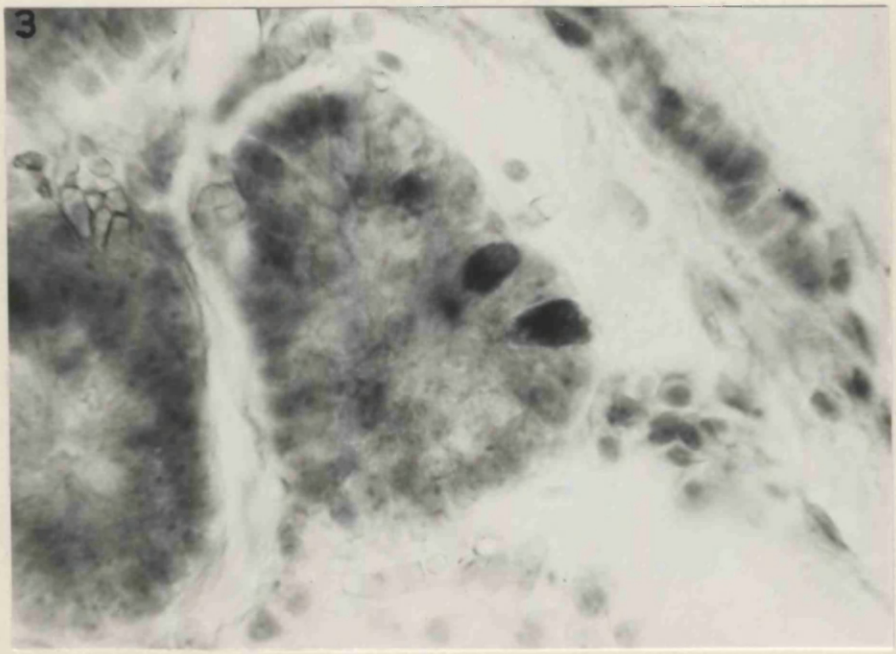
4½ hours after 'skim-cream'.
Sudan IV with Haemalum.
Magnification x 600

High power view; cross section of crypt.

Granular, basophilic epithelial cells near base of crypt.

GUINEA PIG PLATE 2 FIGURES 3

JEJUNUM UPPER THIRD



X 600

Guinea-Pig : Plate 3 : Figures 4 - 5.

Figure 4: Jejunum.

1½ hours after 'skim-cream'.
Sudan IV with Haemalum.
Magnification x 500.

Part of villus tip.

Note: Grading of sudanophilic droplet inclusion size.

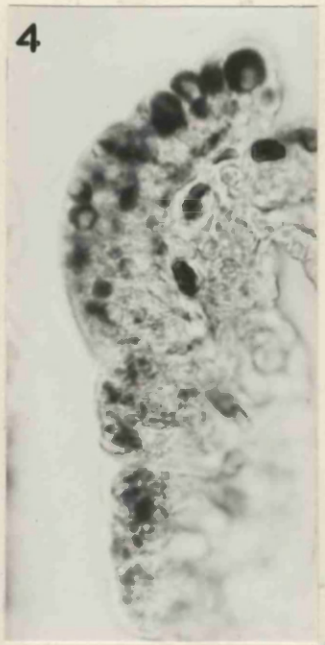
Figure 5: Jejunum.

3 hours after 'skim-cream'.
Sudan IV with Haemalum.
Magnification x 500.

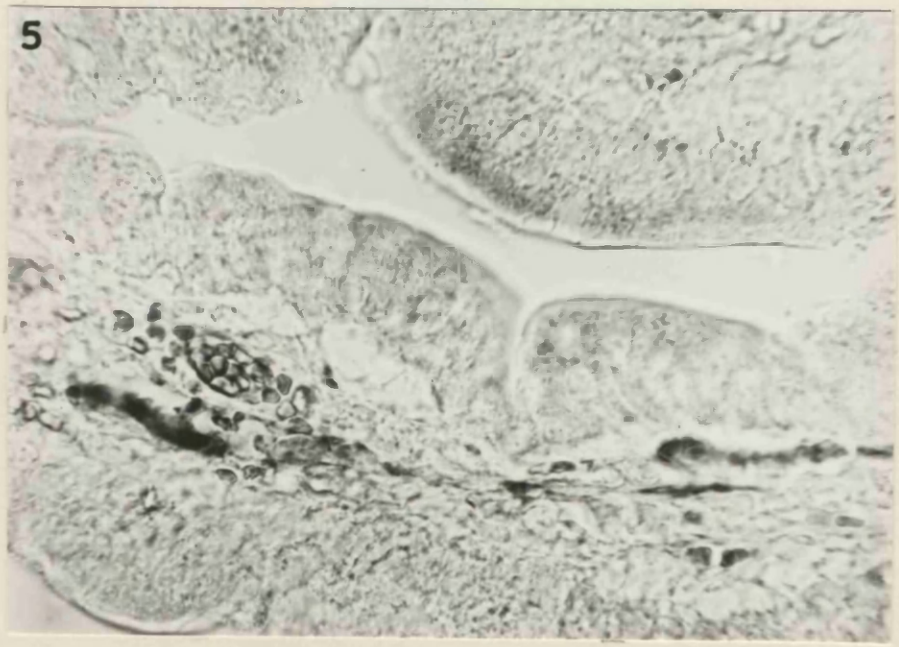
High power view of Villus.
Central lacteal containing free sudanophilic material.
Very fine supranuclear, sudanophilic 'dust'.

GUINEA PIG PLATE 3 FIGURES 4 & 5

JEJUNUM



X 500



X 500

Guinea-Pig : Plate 4 : Figure 6.

Figure 6: Jejunum.

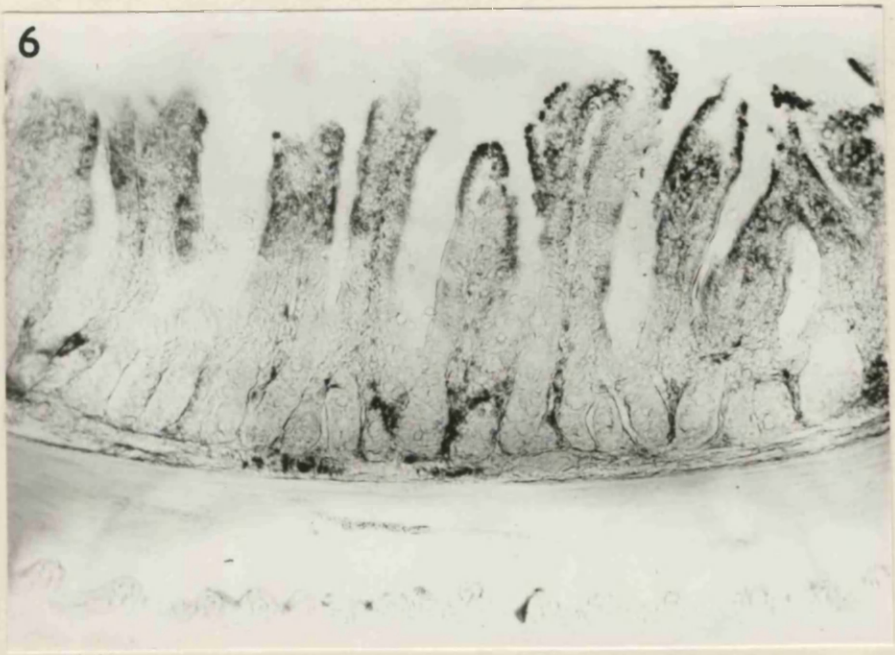
4½ hours after 'skim-cream'.
Sudan IV.
Magnification x 80.

Sudanophilic droplets in epithelium, especially at tips of villi.

Lacteals between crypts joining submucosal lymph vessels.
(Migratory cells in lamina propria).

GUINEA PIG PLATE 4 FIGURE 6

JEJUNUM



X 80

Guinea-Pig : Plate 5 : Figure 7.

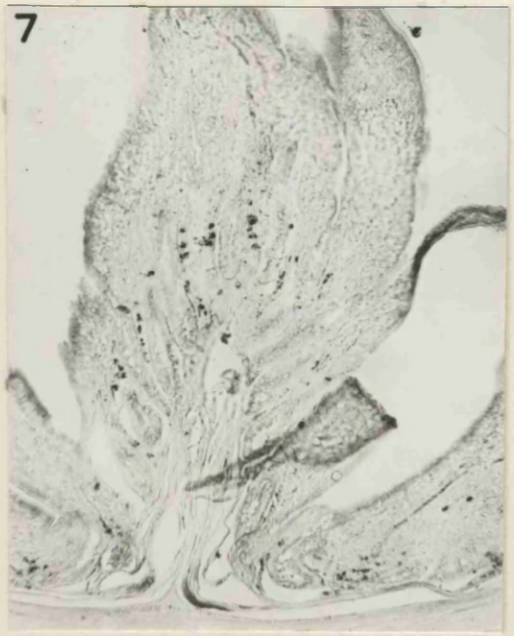
Figure 7: Terminal Ileum.

1½ hours after 'skim-cream'.
Sudan Black.
Magnification x 80.

Invasion of lamina propria between crypts by Sudan-stained granulocytes.

GUINEA PIG PLATE 5 FIGURE 7

TERMINAL ILEUM



X 80

Figure 8: Caecum.
Ordinary Diet.
P.A.S. with Delafield's Haematoxylin.
Magnification x 150.

P.A.S. medium-positive reaction in granularity of luminal aspect of surface cells.
Strongly positive goblet cells in base of crypts (a)
Groups of large mononuclear phagocytic cells (in lamina propria between crypts) with strongly positive granules in cytoplasm (b).

Figure 9: Caecum.
Ordinary Diet.
Sudan Black with Haemalum.
Magnification x 600.

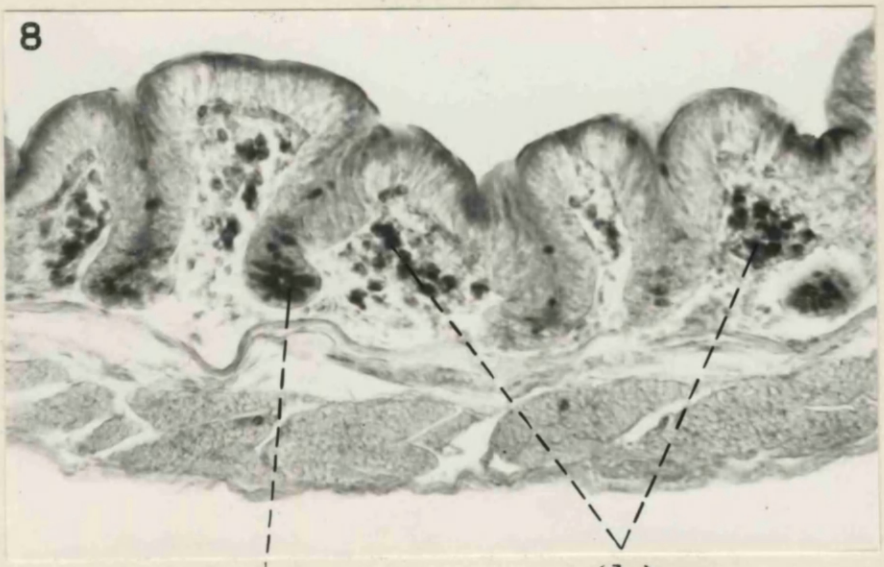
Cross section of crypts and intervening stroma.
Sudan stained inclusions in these large macrophage cells.

Figure 10: Caecum.
Ordinary Diet.
Sudan Black.
Magnification x 150.

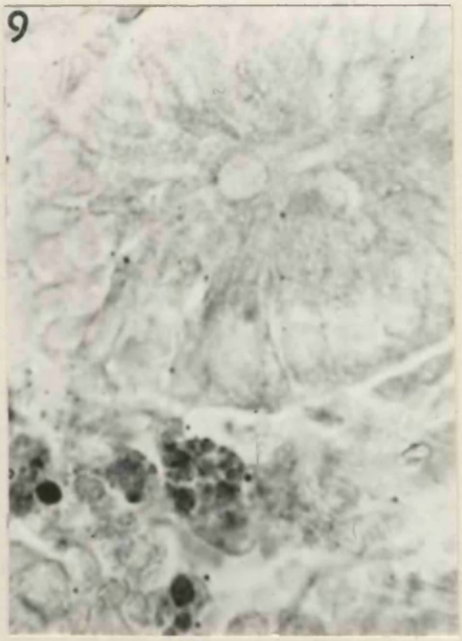
Section of mucosa as in Figure 8.
Large cells in Lamina propria are only faintly tinted with Sudan Black.
Palely tinted granularity in luminal aspect of epithelial cells.

GUINEA PIG PLATE 6 FIGURES 8 - 10

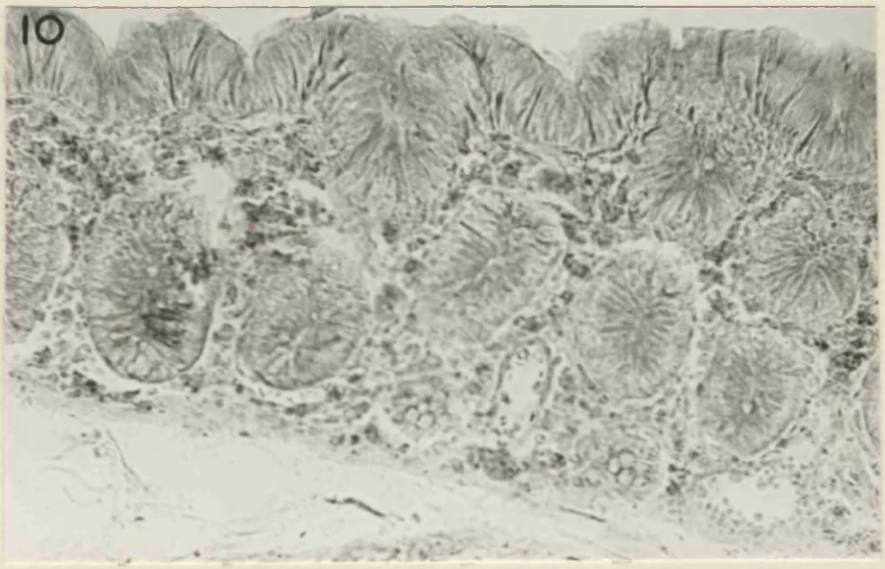
CAECUM



X 150



X 600



X 150

BIBLIOGRAPHY

BIBLIOGRAPHY.

Althausen,T.L. (1950). Diagnosis & Treatment of functional indigestion. Am.Pract. & Digest.Treat. I., 46-49.

Althausen,T.L., Doig,R.K., Uyeyama, K., & Weiden,S.(1950) Digestion & Absorption after massive resection of the small intestine. II. Recovery of the Absorptive function as shown by Intestinal Absorption Tests in two patients and a consideration of compensatory mechanisms. Gastroenterology 16, 126-139.

Althausen,T.L., Uyeyama,K., & Simpson,R.G. (1949). Digestion & Absorption after massive resection of the small intestine. I. Utilisation of food from a "natural" versus a "synthetic" diet, and a comparison of Intestinal Absorption Tests. Gastroenterology 12, 795-807.

Altmann, (1894) Die Elementarorganismen. Leipzig. Cited by Watney,M.R. (1876) in Philos.Trans. 166, 451-488.

André, C. & Favre, M. (1906). Recherches sur l'absorption des Savons.par la Muqueuse Intestinale. J. Physiol.Path.Gèn., 8, 819-822.

Angevine,R.W. (1929). Fat Excretion. vi.Excretion by Thiry-Vella Fistulas. J. biol. Chem. 82, 559-565.

Arnschink,L. (1890). Versuche über die Resorption verschiedener Fette aus dem Darmkanale. Z. Biol. 26, 434-451.

Arnstein,C. (1867).Ueber Becherzellen und ihre Beziehung zur Fettresorption und Sekretion. Virchows Arch. 39, 527-547.

Artom,C. & Peretti,G. (1935). Les Lipides de la Muqueuse intestinale pendant l'absorption de graisses iodées. Arch. int. Physiol. 42, 61-94.

Artom,C., Sarzana,G. & Segré,F. (1937). Influence des graisses Alimentaires sur la formation des Phospholipides dans les Tissus Animaux. Arch. int. Physiol. 47, 245-276.

Asellius,G. (1628). De Lactibus sive Lacteis Venis, Quarto Vasosum Mesaraicorum Genere Novo Invento. J. Baptam Biddellium, Mediolani. Milano.
Cited by Drinker, C.K. & Yoffey,J.M. (1941) in Lymphatics, Lymph & Lymphoid Tissue. p.193. Cambridge, Mass.;Harvard.

Auld,W.H.R. & Needham,C.D. (1951). Experiments on Fat Absorption in a case of Chylothorax. Lancet I, 991-993.

Bacsich,P. (1935). On the staining of lipoid granules in leucocytes.
J. Anat., Lond. 70, 267-272.

Baker,J.R. (1944). The Free Border of the intestinal epithelial cell of vertebrates.
Quart.J.micr.Sci. 84, 73-103.

Baker,J.R. (1946). The histochemical recognition of lipine.
Quart.J.micr.Sci. 87, 441-470.

Baker,J.R. (1947). Further remarks on the histochemical recognition of lipine.
Quart.J.micr.Sci. 88, 463-465.

Baker,J.R. (1951). The Absorption of Lipoid by the Intestinal Epithelium of the Mouse.
Quart.J.micr.Sci. 92, 79-86.

Barcroft,J., McAnally,R.A. & Phillipson,A.T. (1943a). Absorption of volatile acids from the Alimentary tract of the sheep and other animals.
J. Exptl.biol. 20, 120-129.

Barcroft,J., McAnally,R.A., & Phillipson, A.T.(1943b). The absorption of sodium ortho-iodo-hippurate from the rumen of lambs. J. Exptl.biol. 20, 132-134.

Barcroft,J., McAnally,R.A., & Phillipson,A.T. (1944a). The production of Acetic, Propionic & Butyric Acids in the Alimentary Canal. Biochem.J. 38, Proceedings of the Biochem Society ii.

Barcroft,J., McAnally,R.A., & Phillipson,A.T. (1944b). Absorption of Acetic, Propionic & Butyric Acids from the Alimentary Canal. Biochem J. 38, Proceedings of the Biochem.Society, iii.

Bargen,J.A. (1937). Medical management of chronic ulcerative colitis. Proc.Roy.Soc.Med. 30, 351-362.

Barnes, R.H., Miller, E.S., & Burr,G.O. (1941). The Absorption & Transport of Fatty Acids across the Intestinal Mucosa. J.biol.Chem. 140, 233-240.

Basch (1870). Die Ersten Chyluswege und die Fettresorption.
Sitz. Wiener Akad. 62.

Becker,G.H., Meyer, J. & Necheles,H. (1950). Fat absorption in young and old age.
Gastroenterology 14, 80-90.

Béguin,F. (1903). L'intestin pendant le jêune et l'intestin pendant la digestion.
Arch. Anat. micr. 6, 385-454.

Berenbaum,M.C. (1954). Staining of bound lipids.
Nature, Lond. 174, 190.

Bergström,S., Borgström,B., Carlsten, A. & Rottenberg,M. (1950).
On the Mechanism of the Intestinal Fat Absorption.
Acta chem. Scand. 4, 1142-1154.

Bergström,S., Borgström,B. & Rottenberg,M. (1952).
Intestinal Absorption and distribution of fatty acids and glycerides in the rat: metabolism of lipids.
Acta physiol. Scand. 25, 120-137.

Bernard, C. (1877). Cited by Frazer,A.C. in Fat absorption in man.
St. Barth.Hosp.Journ., 54. No.2.Feb.1950.

Bernard, K., Ritzel,G. & Hug,E. (1952).
Ueber die Sczernierung von Lipiden in das Darmlumen bei Abwesenheit der Galle.
Helv.physiol.acta 10, 68-73.

Bernard,K., & Scheitlin,E. (1952). Zur Resorption von Mineralöl.
Helv. physiol.acta 10, 54-61.

Billroth,T. (1858). Ueber die Epithelial zellen der Froschzunge, *sowie* über den Bau der Cylinder - und Flimmerepithelien und ihr Verhältniss zum Bindegewebe.
Muller's Archiv. (1858). 159-177.

Bloom,B., Chaikoff,I.L., Reinhardt,W.O., Entenman,C. & Dauben,W.G. (1950).
The Quantitative significance of the Lymphatic Pathway in transport of absorbed fatty acids.
J.biol.Chem. 184, I - 8.

Bloom,B., Chaikoff,I.L., Reinhardt,W.O., & Dauben,W.G., (1951). Participation of Phospholipides in Lymphatic transport of Absorbed fatty acids. J.biol.Chem. 189, 261-267.

Bloor,W.R. (1912). On Fat Absorption. J.biol.Chem. II, 429-434.

Bloor,W.R. (1913). On Fat Absorption.II. Absorption of fat-like substances other than fats. J.biol.Chem. 15, 105-117.

Bloor,W.R. (1922). Fat transport in the Animal Body. Physiol. Rev. 2, 92-115.

Bloor,W.R. (1923). The Fatty Acids of Blood Plasma. J.biol.Chem. 56, 711-724.

Bloor,W.R. (1943). Biochemistry of Fatty Acids. Reinhold Pub.Corp.; New York.

Bondi,S. & Neumann,A. (1910). Ueber den Weg der Fetteilchen im Blute; ein Beitrag nur Topographie das Fettstaffwechsels. Wien.klin.Wschr. 23, 734-736.

Borgström, B. (1952a). On the mechanism of the intestinal fat absorption. Metabolism of lipids 4. Acta physiol.Scand. 25, 140-149.

Borgström, B. (1952b). On the action of pancreatic lipase on triglycerides in vivo and in vitro. Acta physiol.Scand. 25, 328-347.

Borgström, B. (1953). On the mechanism of the intestinal fat absorption. V. The effect of bile diversion on fat absorption in the rat. Acta physiol.Scand. 28, 279-286.

Borrow, A. & Platt, B.S. (1950). (Personal Communication).

Boyd, E.M. (1935). The reaction of lipids in the blood leucocytes to fever and infection. Surg. Gynec.Obstet. 60, 205-213.

Boyd, F.D., & Robertson, J. (1906). Rectal Alimentation. Scottish Medical & Surgical Journal, 193-216.

Bradley, H.C. (1912). Lipase and fat of Animal Tissues. J.biol.Chem. 13, 407-408.

Bradley,H.C., & Gasser,H.S. (1912). Intestinal Absorption.
J.biol.Chem. II, xx.

Brien,F.S., Turner, D.A., Watson,E.M., & Geddes,J.H.(1952). Study of carbohydrate and fat absorption from normal and diseased intestine in man.
Gastroenterology, 20, 294-297.

Brown,W.L. (1911). An inquiry into the value of Rectal feeding.
Trans.Roy.Soc.Med., Therapeut.Section., 63-76.

Bruce,H.M., & Parkes,A.S. (1949). A complete cubed diet for mice and rats.
J. Hyg., Camb., 47, 202-208.

Bruch,C. (1853). Beiträge zur Anatomie und Physiologie der Dünndarmschleimhaut.
Z.wiss,Zool. 4, 282-298.

Brücke, E. (1851). Ueber ein in der Darmschleimhaut aufgefundenes Muskelsystem.
Sitzungsber.der Wien.Akad.d.Wiss. Kl.II,6, 214-219.

Bunting, C.H., & Huston,J. (1921). Fate of the Lymphocyte.
J.exp.Med. 33, 593-600.

Bunting,C.H. (1922). The Leukocytes.
Physiol.Rev. 2, 505-520.

Bunting,C.H. (1938). The Polymorphonuclear Neutrophile Leucocyte. In Handbook of Hematology Ed. by H. Downey, London.
Vol.I. 151-177

Casey,A.E. (1940). The Diurnal levels of Blood Leukocytes in the normal Rabbit.
Proc.Soc.Exper.Biol.& Med. 45, 863-866.

Chaikoff,I.L., Bloom,B., Steven,B.P., Reinhardt,W.O. & Dauben,W.G. (1951). Pentadecanoic Acid - 5 - C14: Its Absorption and Lymphatic Transport.
J.biol.Chem. 190, 431-435.

Channon,H.J. (1925). Cholesterol Synthesis in the Animal Body.
Biochem. J. 19, 424-432.

Channon,H.J. (1926). The biological significance of the unsaponifiable matter of oils. I.Experiments with the unsaturated hydrocarbon, Squalene. Biochem.J. 20, 400-408.

Channon,H.J. (1928). The biological significance of the unsaponifiable matter of oils. III. Fish-liver oils. Biochem.J. 22, 51-59.

Channon,H.J., & Collinson,G.A. (1928). The biological significance of the unsaponifiable matter of Oils. IV. The absorption of Higher Alcohols. Biochem.J. 22, 391-401.

Channon,H.J. & Collinson,G.A. (1929). The Unsaponifiable fraction of liver oils. V. The Absorption of liquid paraffin from the Alimentary Tract in the Rat and the Pig. Biochem.J. 23, 676-688.

Channon,H.J. & Devine,J. (1934). The Absorption of n-hexadecane from the Alimentary Tract of the Cat. Biochem.J. 28, 467-471.

Channon,H.J., Devine,J., & Loach,J.F. (1934). The hydrocarbons of the Pig Liver. Biochem.J. 28, 2012-2025.

Channon,H.J. & Marrian,G.F. (1926). The biological significance of the unsaponifiable matter of oils. II. An unidentified unsaturated hydrocarbon present in mammalian liver. Biochem.J. 20, 409-418.

Ciaccio,C. (1910). Contributo alla conoscenza dei lipoidi cellulari. Anat.Anz. 35, 17-31.

Clark,E.R. & Clark,E.L. (1917). A study of the reaction of lymphatic endothelium and leucocytes in the tadpole's tail, toward injected fat. Amer.J.Anat. 21, 421-448.

Clark,E.R. & Clark,E.L. (1930a). Observations on the Macrophages of living Amphibean Larvae. Amer.J.Anat. 46, 91-148.

Clark,E.R. & Clark,E.L. (1930b). Relation of monocytes of the blood to the tissue macrophages. Amer.J.Anat. 46, 149-184.

Clarke, B.G., Ivy,A.C. & Goodman,D. (1948). Effect of resection of mesenteric lymph nodes on intestinal fat absorption in the Dog. Amer.J.Physiol. 153, 264-267.

Cooke,W.T., Elkes,J.J., Frazer,A.C., Parker, J., Peeney,A.L.P., Sammons,H.G. & Thomas,G.(1946). Anomalies of intestinal absorption of fat. I. Determination and significance of faecal fat. Quart.J.Med. 15, 141-155.

Cook,R.P. & Thompson,R.O. (1951). The absorption of fat and of cholesterol in the rat, guinea-pig and rabbit. Quart.J.exp.Physiol. 36, 61-74.

Cramer,W. & Ludford,R.J. (1925). On cellular changes in Intestinal fat absorption. J.Physiol. 60, 342-346.

Cruikshank, (1790). The Anatomy of Absorbing Vessels. London. Cited by Watney,H.R. (1876) in The minute anatomy of the Alimentary Canal. Philos.Trans. 166, 451-488.

Cuneo,M. & Delamare,G. (1900). Note sur l'histologie des lymphatiques de l'estomac. Compt.rend.Soc.de Biol. 52, 428-429.

Curry,F.S. & Bargen,J.A. (1935). Studies on absorption and excretion in segments of the colon in man. Surg.Gynec.Obstet.60, 667-673.

Czerny,V. & Latschenberger,J. (1874). Physiologische Untersuchungen uber die Verdauung und Resorption im Dickdarm des Menschen. Virchows Arch. 59, 161-190.

Czerney (1907). Zur Kentiss der exudation Diathese. Mschr.Kinderheilk. 6, 1.

Dabelow,A. (1930). Reaktionsweisen des Lymphknotens beim Fetttransport (unter besonderer Berücksichtigung des Mesenterialknotens). Z. Zellforsch. 12, 207-273.

Danchakoff,V. & Seidlein,S.M. (1922). Digestive activity of mesenchyme and its derivatives.II. Proteins as object. Biol.Bull.Lancaster,Pa. 43, 97-122.

Debove. (1872). Cited by Watney,H.R. (1876) in "The Minute Anatomy of the Alimentary Canal". Philos.Trans. 166, 451-488.

Deucher,P. (1897). Über die Resorption des Fettes aus Klystieren. Deut.Arch.für klin.Med. 58, 210-236.

Dobson,M.J. (1955). A histological study of the structure and organization of the rumen epithelium of sheep. J.Physiol. 128, P. 1.

Donders, (1856). Physiologie Deutch von Theil I. Cited by Watney,H.R.(1876) in "The Minute Anatomy of the Alimentary Canal". Philos.Trans. 166, 451-488.

Donitz,W. (1864). Ueber die Schleimhaut des Darmkanals. Arch.Anat.Physiol., Lpz., 367-406.

Doubilet,H. & Reiner,M. (1937). Absorption of fat from the ileum in human beings. Arch.intern.Med. 59, 857-864.

Drago, U. (1901). Changements de forme et de structure de l'épithélium intestinal durant l'absorption des graisses. Arch.ital.Biol. 35, 300.

Drinker,C.K. & Yoffey,J.M. (1941). Lymphatics, Lymph & Lymphoid Tissue. Cambridge, Massachusetts; Harvard University Press.

Eberth, C.J. (1861). Zur Entstehung der Schleimkörper. Virchows Arch. 21, 106-115.

Eberth, C.J. (1864). Ueber den Fieneren Bau der Darmschleimhaut. Würzb.Naturw.Zeitschr. 5.

Eckstein, H.C. (1925). The fatty acids in the subcutaneous fat of man. J.biol.Chem. 64, 797-806.

Ehrenthal, W. (1891). Arch.Physiol. 48, 75. Cited by Angevine,R.W. (1929) in Excretion by Thiry-Vella Fistulas. J.biol.Chem. 82, 559-565.

Ehrlich,P. (1879). Ueber die specifischen Granulationen des Blutes.
Arch.Anat. Physiol. Lpz., Physiol.Abt.,571.

Eimer, T. (1869) Die Wege des Fettes in der Darmschleimhaut bei seiner Resorption.
Virchows Arch. 48, 119-176.

Elkes, J.J., Frazer, A.C. & Stewart, H.C. (1939). The Composition of particles seen in normal human blood under dark-ground illumination.
J. Physiol. 95, 68-82.

Elkes, J.J., Frazer, A.C., Schulman,J.H., & Stewart,H.C. (1944). The mechanism of emulsification of triglyceride in the small intestine.
J. Physiol. 103, 6P-7P.

Elkes, J.J., Frazer, A.C., Schulman,J.H., & Stewart,H.C. (1945). Preferential adsorption of proteins at charged oil/water interfaces.
Proc.Roy.Soc.A. 184, 102-115.

Elsden,S.R. & Phillipson,A.T. (1948). Ruminant Digestion.
Annu.Rev.Biochem. 17, 705-726.

Eränkö,O. (1950). Demonstration of Glycogen and Lipids in the Cytoplasm of Human Neutrophilic Leucocytes.
Nature,Lond. 165, 116-117.

Erdmann. (1867). Beobachtungen über die Resorptionswege in der Schleimhaut des Dünndarms.
Inaug. Dissert.Dorpat.
Cited by Watney, H. (1876) in "The Minute Anatomy of the Alimentary Canal".
Philos. Trans. 166, 451-488.

Evans,H.M. (1915). The macrophages of mammals.
Amer.J.Physiol. 37, 243.

Ewald,C.A. und Boas. (1883). Beiträge zur Physiologie und Pathologie der Verdanung.
Virchows Arch. 104, 302.

Exner, S. (1901). Ueber die Resorption künstlich gefärbter Fette.
Pflüg.Arch. ges Physiol. 85, 628-635.

Exner, A. (1903). Über die durch intraperitonale Adrenalin injektion verursachte Verzögerung der Resorption von in den magen eingeführten giften.
Arch.exp.Path. Pharmak. 50, 313-318.

Fairley,N.H. & Mackie, F.P. (1937). The clinical & biochemical syndrome in lymphadenoma and allied diseases involving the mesenteric lymph nodes.
Brit.med.J.I. 375-380.

Favarger, P. & Collett,R.A.(1950). Estimation du degré d'hydrolyse des graisses pendant la résorption intestinale.
Helv.Physiol.et Pharmacol. Acta 8, C15-16.

Fressinger,N. et Marie,P.L. (1909). Les ferments des leucocytes dans les exudats des séreuses.
C.R. de l. Soc. de Biol., 1962.

Fischler,F. (1904). Ueber die Unterscheidung von Neutralfetten, Fettsäuren und Seifen im gewebe.
Centralbl.Allg.Path.und Path.Anat.15, 913-917

Fischler,F. (1925). Ist der Traubenzucker ein Hormon?
Centralbl.Allg.Path.und Path.Anat.36,293-294.

Fles (1865) . Onderzoekingen over de Histologische Zamenstelling des vlokjes van het Darmkanal. M.Hand.t.d. Stelselm outleedkunde v.d. Mensch. Cited by Watney, H. (1876) in "The Minute Anatomy of the Alimentary Canal". Philos. Trans. 166, 451-488.

Flower,W.H. (1872). Lectures on the Comparative Anatomy of the Organs of Digestion of the Mammalia. Medical Times & Gazette, I. 215-219.

Fohman (1827). Das Saugadersystem der Wirbelthiere, Heidelberg.
Cited by Watney,H. (1876) in "The Minute Anatomy of the Alimentary Canal".
Philos.Trans. 166, 451-488.

Frank,O. (1898). Zur Lehre von der Fettresorption.
Z.Biol. 36, 568-593.

Frank,O. & Ritter,A. (1906). Einwirkung der Überlebenden Dünndarmschleimhaut auf Seifen, Fettsäuren und Fette.
Z.Biol. 47, 251-267.

Frazer, A.C. (1938). Fat Absorption and Metabolism.
The Analyst, 63. 308-313.

Frazer, A.C. (1940). Fat Absorption and Its Relationship to Fat Metabolism.
Physiol.Rev. 20, 561-581.

Frazer, A.C. (1943a). Differentiation in the Absorption of Olive Oil and Oleic Acid in the rat.
J. Physiol. 102, 306-312.

Frazer, A.C. (1943b). Lipolysis and Fat absorption.
J. Physiol. 102, 329-333.

Frazer, A.C. (1946). The Absorption of Triglyceride Fat from the Intestine.
Physiol.Rev. 26, 104-119.

Frazer, A.C. (1947). Aetiology of Steatorrhoea.
Brit. med.J. 2, 641-645.

Frazer, A.C. (1949a). Effect of Choline on the Intestinal Absorption of fat.
Nature,Lond., 157. 414.

Frazer, A.C. (1949b). Fat Metabolism and the Sprue Syndrome.
Brit.Med.J. 2, 769-773.

Frazer, A.C. (1950). Fat absorption in man.
St. Barth.Hosp.Journal, 54. No.2. (Feb., 1950).

Frazer, A.C. (1951a). Le système d'absorption des graisses.
Bull.Soc.Chim.biol.,Par.33, 961-967.

Frazer, A.C. (1951b). Les vitamines et l'absorption des graisses.
Bull.Soc.Chim.biol.,Par.33, 968-972.

Frazer, A.C. (1952). Fat Metabolism.
Annu.Rev.Biochem. 21, 245-272.

Frazer, A.C, Elkes, J.J., Sammons, H.G., Govan,A.D.T., & Cooke, W.T. (1945). Effects of Cl.Welchii Type A Toxin on body tissues and fluids.
Lancet I, 457-460.

Frazer, A.C., French, J.M., Sammons,H.G.,Thomas,G. & Thompson,M.D. (1949a). The Quantitative & Qualitative Investigation of fat absorption. Brit.J.Nutr. 3, 358-363.

Frazer, A.C,, French,J.M., Sammons,H.G., Thomas,G. & Thompson,M.D. (1949b). Normal and abnormal fat absorption in the human subject and its nutritional significance. Brit.J.Nutr. 3, 363-367.

Frazer,A.C., & Sammons,H.G. (1944). The relationship of lipolysis to emulsification of triglyceride in the small intestine. J.Physiol. 103, 5P-6P.

Frazer,A.C., & Sammons,H.G. (1945). The formation of Mono- and Di-glycerides during the Hydrolysis of Triglyceride by pancreatic lipase. Biochem J. 39, 122-128.

Frazer,A.C., Schulman,J.H., & Stewart,H.C. (1944). Emulsification of fat in the intestine of the rat and its relationship to absorption. J.Physiol. 103, 306-316.

Frazer,A.C., & Stewart,H.C. (1937). Ultramicroscopic particles in normal human blood. J.Physiol. 90, 18-30.

Frazer,A.C. & Stewart,H.C. (1942). Emulsification and Absorption of fats and paraffins in the intestine. Nature,Lond. 149, 167-168.

Frazer,A.C. & Walsh,V.G. (1933). An apparatus for the production of finely dispersed emulsions, and the rate of digestion of fat by lipase in relation to the surface area. J.Physiol. 78, 467-474.

Freeman,L.W. & Johnson,V. (1940). The hemolytic action of chyle. Amer.J.Physiol. 129, p. 357-358.

Frerichs. (1846). Handworterbuch der Physiologie,von Dr. Wagner. Artikel: Verdauung.3. Cited by Watney,H. (1876) in Philos.Trans. 166, 451-488.

Friedenthal,N. (1900). Ueber die bei der Resorption der Nahrung in Betracht kommenden Krafte. Arch.Anat.Physiol., Lpz., Physiol.Abstr. 217-281.

Friedreich (1858). Einiges über die Struktur der Cylinder -nnd Flimmer-epithelien. Virchows Arch. 15.

Fries,E. (1867). Ueber die Fettresorption und die Entstehung der Becherzellen. Virchows Arch. 40, 519-531.

Funke, O. (1855). Beitrage Physiol. der Verdauung. Z.wiss Zool. 7, 315.

Gage,S.H. & Gage, S.P. (1890). Changes in ciliated areas of alimentary canal of amphibia. Proc.Amer.Assoc.Adv.Sci. 39, 337-338.

Gage,S.H. & Fish,P.A. (1924). Fat Digestion, Absorption, and Assimilation in man & animals as determined by the dark-field microscope, and a fat-soluble dye. Amer.J.Anat. 34, I-86.

Gardner,J.A. (1921). On the Composition of the Unsaponifiable matter of the Ether Extract of Human Faeces. Biochem.J. 15, 244-273.

Gardner,J.A. & Gainsborough,H. (1930). Blood cholesterol studies in biliary & hepatic disease. Quart.J.Med. 23, 465-483.

Garrey,W.E. & Butler,Virginia (1932). The digestive leucocytosis question. Amer.J.Physiol. 100, 351-356.

Glynn,L.E. & Rosenheim,M.L. (1938). Mesenteric chyladenectasis with steatorrhoea and features of Addison's disease. J.Path.Bact. 47, 285-290.

Goldmann,J.von (1933). Über die Lipoidsubstanzen in Zellen mesenchymaler Herkunft. Virchows Arch. 287, 587-601.

Goodsir,J. (1842). On the ultimate secreting structure, and on the laws of its function. Trans.R.S. Edinb., 15, 295-305.

Greene,C.W. & Skaer,W.F. (1911). Absorption of fat by the Mammalian Stomach. Amer.J.Physiol. 29, Proc.Amer.Physiol. Soc. xxxvi - viii.

Greene,C.W. & Skaer,W.F. (1913). Evidence of fat absorption by the mucosa of the Mammalian Stomach.
Amer.J.Physiol. 32, 358-368.

Gregory,R.A. (1954). The Digestive System.
Annu. Rev.Physiol. 16, 155-174.

Gruby & Delafond (1843). Anatomie et fonctions des villosités intestinales.
Comptes Rendus Acad.Sci., Paris. 16.

Gruenhagen,A. (1887). Über Fettresorption im Darme.
Anat.Anz. 2, 424-425.

Gruenhagen,A. (1889). Über Fettresorption im Darmepithel.
Arch.mikr.Anat. 29, I.

Gruenhagen,A. & Krohn, (1889).
Pflüg.Arch.ges.Physiol. 44, 535 .

Grützner,P.von (1898). Ueber die Bewegungen des Darminhaetes.
Pflüg.Arch.ges.Physiol. 71, 492-522.

Grützner,P.von (1905). Ein Beitrag zum Mechanismus der Magenverdauung.
Pflug.Arch.ges Physiol. 106, 463-522.

Gumilewski (1886).
Pflug.Arch.ges Physiol. 39, 556.
Cited by Angevine,R.W. (1929) in Excretion by Thiry Vella Fistulas.
J.Biol.Chem. 82, 559-565.

Hamburger,H.J. (1900). Over de resorptie von vet en zeep in den dikken en dunnen darm.
Nederl.Tijdschr.v.Geneesk.,Amst., 36. 727-737

Hardy,W.B. & Wesbrook,F.F.(1895). The wandering cells of the Alimentary Canal.
J.Physiol. 18, 490-524.

Heidenhain,M. (1899). Ueber die Struktur der Darmepithelzellen.
Arch.mikr.Anat. 54, 184-224.

Heidenhain,R. (1858). Die Absorptionswege des Fettes.
Moleschott's Unters.z.Naturl.des Menschen und der Thiere. IV.

Heidenhain,R. (1888). Beiträge zur Histologie und Physiologie der Dünndarmschleimhaut. Pflüg.Arch.ges Physiol. 43, Suppl.I-82.

Heidenhain,R. (1891). Versuche und Fragen zur Lehre von der Lymphbildung. Pflüg.Arch.ges Physiol. 49, 209-301.

Heidenhain,R. (1907). Plasma und Zelle. Bardelebon's Handbook of Anatomy, Jena, 1907.

Henle,J. (1838). Über die Fortschritte des physiologischen Pathologie und pathologischen Anatomie. Müllers Arch. (1838). Supplement, i - cxcvii.

Henle,J. (1841). Allgemeine Anatomie, Leipzig. Cited by Baker, J. in The Free border of the intestinal epithelial cell of vertebrates. Quart.J.Micr.Sci. 84, 73.

Henriques,V. & Hansen,C. (1900). Vergleichende unter-Suchungen über die chemische Zusammensetzung des thierischen Fettes. Skandin.Arch.f.Physiol, Leipz.(1901) 11, 151-165.

Hermann,L. (1890). Arch.ges Physiol. 46, 93.
Cited by Angevine,R.W. (1929) in Excretion by Thiry-Vella Fistulas.
J.biol.chem. 82, 559-565.

Hewson (1846). The works of. Printed by Sydenham Society. Ed. by Gulliver,G.

Hill,Elsie & Bloor,W.R. (1922). Fat excretion.
J.biol.Chem. 53, 171-178.

Hill, J.M. (1937). Mesenteric Chyladenectasis.
Amer.J.Path. 13, 267-275.

Hirayama,S. (1922). Experimental researches on the absorption of fat in the stomach.
Japanese Medical World 2, 101-106.

His, W. (1862). Untersuchungen über den Bau der Peyer'schen Drüsen und der Darmschleimhaut.
Z.wiss.Zool. II, 416-442.

His,W. (1863). Ueber das Epithel der Lymphgefässwurzeln und über die v. Recklinghausen'schen Saftcanälchen.
Z.wiss.Zool. 13, 455-473.

Hofbauer,L. (1900). Kann Fett unverseift resorbirt werden? Eine Versuchsreite zur Beantwortung dieser Frage. Pflüg.Arch.ges Physiol. 81, 263-266.

Holmes,A.D. & Kerr,R.H. (1923). Notes on the ether extract of faeces. J.biol.Chem. 58, 377-381.

Holthusen,H. (1910). Über den histologischen Nachweis verschiedener Fetterten mit Rücksicht auf das Verhalten des Fettes in den Lymphknoten. Beitr.path.Anat. 49, 595.

Home,E. (1813). On the formation of fat in the intestines of living animals. Philos.Trans. 103, 146-158.

Honigmann,G. (1896). Beiträge zur Kenntnis der Aufsaugungs und Ausscheidungsvorgänge im Darme. Arch.VerdauKr. 2, 296-320.

Hull,M. & Keeton,R.W. (1917). The existence of a gastric lipase. J.biol.Chem. 32, 127-140.

Hutchison,H.S. (1919). Fat metabolism in health & disease with special reference to infancy & childhood.
Quart.J.Med. 13, 277-292.

Irwin,M.H., Steenbock,H. & Templin,V.M. (1936). The influence of vitamins A,B, or D, anemia or fasting upon the rate of fat absorption in the rat.
J.Nutr. 12, 357-364.

Jackson,C.M. (1913). Postnatal growth and variability of the Body and of the various organs in the albino rat.
Amer.J.Anat. 15, 1-68.

Jackson,C.M. (1915). Changes in the relative weights of the various parts, systems and organs of the young albino rats held at constant body weight by underfeeding for various periods.
J. exper.Zool. 19, 99-156.

Jackson,C.M. (1915). Effects of acute and chronic inanition upon the relative weights of the various organs and systems of adult albino rats.
Amer.J.Anat. 18, 75-116.

Jeker, L. (1936). Mikroskopische Untersuchungen über die Fettresorption im normalen darm und bei Hemmung der Fettresorption durch Monojodessigsäure und Phlorrhizin.
Pflüg. Arch. ges Physiol. 237, 1 -13.

Johnson,V. & Freeman,W. (1938). The adaptive value of absorption of fats into the lymphatics.
Amer.J.Physiol. 124, 466-469.

Jones,I.D. (1924). Is lymphatic obstruction a factor in sprue?
Lancet, 2. 525.

Jones,C.M., Culver P.J., Drummey,G.D., & Ryan,A.E. (1948). Modification of fat absorption in digestive tract by use of emulsifying agent.
Ann.intern.Med. 29, 1-10.

Jordan,H.E. & Speidel,C.C. (1923). Studies on lymphocytes. I. Effect of splenectomy, experimental haemorrhage and a haemolytic toxin in the frog.
Amer.J.Anat. 32, 155-187.

Jordan,H.E. & Speidel,C.C. (1924). Studies on lymphocytes. III. Granulocytopoiesis in the salamander, with special reference to the monophyletic theory of blood-cell origin.
Amer.J.Anat. 33, 485-505.

Kaufmann,C. & Lehmann,E. (1928). Über den histochemischen Fettrachweis im Gewebe. Untersuchungen unter besonderer Berücksichtigung des von Ciaccio Angegebenen Färbeverfahrens.
Virchows Arch. 270, 360-398.

Kaywin,L. (1936). Cytological study of digestive system of anuran larvae during accelerated metamorphasis.
Anat.Rec. 64, 413-437.

Kennon,B.R., Shipp,M.E., & Hetherington,D.C.(1937). Study of white blood cell picture in 6 young men.
Amer.J.Physiol. 118, 690-696.

Kiddle,P., Marshall,R.A. & Phillipson,A.T. (1951). A comparison of the mixtures of acetic, propionic and butyric acids in the rumen and in the blood leaving the rumen.
J.Physiol. 113, 207-217.

Kischensky,D.P. (1902). Über die Resorption des Fettes im Darmkanal.
Beitr.path.Anat. 32, 197.

Klein,A. & Porter, W.B. (1944). Intestinal Malabsorption associated with tuberculosis of mesenteric lymph nodes.
Arch.intern.Med. 74.

Klemperer, G. & Scheurlen,E. (1889). Das Verhalten des Fettes im Magen.
Z. klin.Med. 15, 370-378.

Kobert,R. & Koch,W. (1894). Einiges über die Funktionen des menschlichen Dickdarms.
Dtsch.med.Wschr. 20, 883.

Kölliker,A.von (1856). Besonderer Banes der Cylinderzellen des Dünndarms.
Verh.der Phys.Med.ges.Würzburg, 6.

Krakower,A. (1934). 'Faecal fat' and its relation to fat in the diet.
Amer.J.Physiol. 107, 49-54.

Krause, C. (1837). Beobachtungen und Bemerkungen.
Müllers Arch. 1-36.

Krehl,L. (1890). Ein Beitrag zur Fettresorption.
Arch.Anat.Physiol. 97-112.

Lacauchie (1843). Cited by Watney,H.R. (1876) in
The Minute Anatomy of the Alimentary Canal.
Philos.Trans. 166, 451-488.

Lamb, F.W. (1910). Some Observations on Fat Absorption.
J. Physiol. 40, xxiii-xxvi.

Leach,E.H. (1938). The role of leucocytes in fat absorption.
J. Physiol. 93, 1-9 .

Lefholz,R. (1923). The effects of diets varying in calorie value and in relative amounts of fat, sugar and protein upon the growth of lymphoid tissue in kittens.
Amer.J.Anat. 32, 1-35 .

Letzerich, L. (1866). Ueber die Resorption der verdauten Nährstoffe im Dünndarm.
Virchows Arch. 37, 232-252.

Levites,S. (1906). Über die Verdauung der Fette im Tierischen organismus.
Z.phys.Chem. 49, 273-285.

Leydig (1866). Traité d'histologie. Paris: 1866. Cited by Baker in The free border of the intestinal epithelial cell of vertebrates.
Quart.J.Micr.Sci. 84, 73.

Lieberkuhn, (1745). De fabrica et actione villorum. Amstelod. Cited by Watney,H.R.(1876) in Philos. Trans. 166, 451-488.

Lison,L. (1936). Histochemie Animale. Paris: Gauthier-Villars.

Little,J.M. & Robinson,C.S.(1941). The transportation of absorbed lipids.
Amer.J.Physiol. 134, 773-780.

Loeper,M. & Marchal,G.(1922). La constance de la leucogenèse intragastrique après ingestion de bouillon.
Comptes Rendes Soc.biol. 87, 1081.

Loevenhart,A.S. (1902). On the relation of lipase to fat metabolism - lipogenesis. Amer.J.Physiol. 6, 331-350.

Lorrain-Smith,J. (1908). On the simultaneous staining of neutral fat and fatty acids by oxazine dyes. J.Path.Bact. 12, 1-4 .

Lorrain-Smith,J. & Mair,W. (1909). An investigation of the principles underlying Weigert's method of staining medullated nerve. J.Path.Bact. 13, 14-27.

Lundbaek,K. & Maaløe,O.(1947). Non-absorption of finely emulsified paraffinum liquidum in the Intestine of the rat. Acta physiol.Scand. 13, 247-252.

Macallum,A.B. (1894). On the absorption of iron in the Animal body. J.Physiol. 16, 268-297.

Mahler,P. & Nonnenbruch,W. (1932). Arbeiten über die Physiologie und Pathologie des Dünndarms. V. Die Fettresorption im Gereizten Darm. Z.ges.exp.Med. 85, 112-114.

Marder,L., Becker,G.H., Maizel,B. & Necheles,H.(1952). Fat Absorption and Chylomicronemia. Gastroenterology 20. 43-59.

Maximow,A. (1902). Experimentelle Untersuchungen über die entzündliche Neubildung von Bindegewebe. Beitr.path.Anat. Suppl.5, 1

Maximow,A. (1927). Bindegewebe und blutbildende gewebe. In W. von Möllendorff's Handbuch der Mikroskopischen Anatomie des Menschen. Vol.2. Pt. 7. 232.

Masson,M.J. & Phillipson,A.T. (1951). The absorption of acetate, propionate and butyrate from the rumen of the sheep. J.Physiol. 113, 189-206.

Masson,M.J. & Phillipson,A.T. (1952). The composition of the digesta leaving the abomasum of the sheep. J.Physiol. 116, 98-111.

McAnally,R.A. & Phillipson,A.T. (1943). Absorption of volatile acids from rumen of the sheep. J.Physiol. 101, Proc.Physiol.Soc. 13p-14p.

McManus,J.F.A. (1945). Granules of the Human Polymorphonuclear Leucocyte.
Nature,Lond. 156, 173.

Mellanby,J. (1927). Petroleum emulsion in the small intestine.
J.Physiol. 64, Proc.Physiol.Soc. xxxiii-xxxiv.

Mendel,L.B. (1909). The Absorption of Fats stained with Sudan III.
Amer.J.Physiol. 24, 493-496.

Mendel,L.B. & Baumann,E.J. (1915). The question of fat absorption from the mammalian stomach.
J.biol.Chem. 22, 165-189.

Mendel,L.B. & Daniels,A.L.(1912). The behaviour of fat soluble dyes & stained fat in the animal organism.
J.biol.Chem. 13, 71-95.

Metschnikoff,E.(1883). Untersuchungen mesodermalen Phagocyten einiger Wirbelthiere.
Biol.Zbl. 3, 560.

Metschinoff,E. (1879). Spongiologische Studien.
Z.wiss.Zool. 32, 349-387.

Minard,F.N. (1953). The inhibition of the action of pancreatic lipase by esters of polyoxyethylene sorbitan.
J.biol.Chem. 200, 657-660.

Moleschott,J. & Marfels,F.(1854). Der Uebergang kleiner gester Theilchen aus dem Darmkanal in den Milchsaft und das Blut.
Wien.med.Wschr. 4, 817-822.

Möller,W. (1899). Anatomische Beiträge zur Frage von der Sekretion und Resorption in der Darmschleimhaut.
Z.wiss.Zool. 66, 69-135.

Moore,B. (1903). On the synthesis of Fats accompanying Absorption from the Intestine.
Proc.Roy.Soc. 72, 134-151.

Morales,S., Chung,A.W., Lewis,J.M., Messina,A. & Holt,L.E.Jr. (1950). Absorption of Fat and Vitamin A in Premature Infants.
Pediatrics 6, 644-649.

Mottram,J.C., Cramer,W. & Drew,A.H. (1922). Vitamins, exposure to radium and intestinal fat absorption.
Brit.J.exp.Path. 3, 179-181.

Müller,F. (1884).
Z.Biol. 20, 327.

Müller,F. (1893).
Virchows Arch. 131, 106.

Munk,I. (1880). Zur kenntniss der Bedeutung des Fettes und seiner Componenten für den Staffwechsel.
Virchows Arch. 80, 10-47.

Munk,I. (1884). Zur Lehre von der Resorption, Bildung und Ablagerung der Fette im Thierkörper.
Virchows Arch. 95, 407-467.

Munk,I. (1900a). Zur Frage der Fettresorption.
Zbl.Physiol. 14, 121-125.

Munk,I. (1900b). Zur Frage der Fettresorption.
Zbl.Physiol. 14, 153-156.

Munk,I. (1900c). Die Frage der Fettresorption und Herr. E.Pflüger.
Zbl.Physiol. 14, 409-412.

Munk,I. & Rosenstein,A. (1891). Zur Lehre von der Resorption im Darm, nach Untersuchungen an einer Lympz-(Chylus)-fistel beim Menschen.
Virchows Arch. 123, 230-484.

Nakashima,K. (1914). Zur Frage der Resorption des Fettes im Dick - und Mastdarm.
Pflüg.Arch.ges.Physiol. 158, 288-306.

~~Neumann (1907).~~

Newell,G.E. & Baxter,E.W.(1936). On the nature of the free cell-border of certain mid-gut epithelia.
Quart.J.micr.Sci. 79, 123-150.

Niemann,A. (1912). Das verhalten des Stoffwechsels bei Angeborenem Verschlub der Gallenwege.
Z.Kinderheilk 4, 152-167.

Noll,A. (1908). Über Fettsynthese im Darmepithel des Frosches bei der Fettresorption.
Arch. Physiol.Lpz. Suppl. 145-160.

Noll,A. (1910). Chemische und Mikroskopische Untersuchungen über den Fettransport durch die Darmwand bei der Resorption.
Pflüg. Arch.ges.Physiol. 136, 208-247.

Nuhn,(1849) Unters. und Beobacht a.d. Gebiete der Anatomie. Heidelberg. Cited by Watney,H. (1876) in Philos.Trans.166, 451-488.

Nunez,C.J. & Bargen,J.A.(1942). Fat Excretion in the bowel of man.
Ann.intern.med. 17, 60-64.

Ogata (1881). Die Zerlegung neutraler Fette im Lebendigen Magen.
Du Bois' Archiv. 515.

Ohno,R. (1930). Biochemische Studien über eine neu Funktion der Lymphocyten in der Darmwand,insbesondere in den Darmfollikeln.
Biochem.Z. 218, 206-246.

Onazaki,T. (1936). Histologische Beiträge zur Frage der Fettresorption aus dem Dickdarm.
Tohoku J.exp.Med.29, 224-243.

Oppel,A. (1899). Verdauungsapparat.
Ergebn.Anat.EntzGesch. 9, 84-164.

Parthasarathy,D., Garton,G.A., Phillipson,A.T.(1952).
The passage of phosphorus across the rumen epithelium of sheep.
Biochem.J. 52, xvi-xvii.

Pearse,A.G.E.(1953). Histochemistry-Theoretical & Applied.
1st.ed. Churchill: London.

Perewoznikoff,(1867). Zur Frage don ver Synthese des Fettes.
Zbl.med.Wiss. 851.

Perlman,I., Ruben,S., & Chaikoff,I.L.(1938). Radioactive Phosphorus as an indicator of phospholipid metabolism.
J.biol.Chem. 122, 169-182.

Pettenkofer,M. & Voit,C.(1873).
Z.Biol. 9, 1.

Pfander,W.H. & Phillipson,A.T.(1953). The rates of absorption of acetic, propronic, & n-butyric acids.
J.Physiol. 122, 102-110.

Pflüger,E. (1900a). Ueber die Resorption knenstlich gefaerbter Fette.
Pflüg.Arch.ges.Physiol. 81, 375-380.

Pflüger,E. (1900b). Der gegenwaertige Zustand der Lehre von der Verdauung und Resorption der Fette und eine *Verurtheilung* der hiermit verknuepften physiologischen vivèsectionen am menschen.
Pflüg.Arch.ges.Physiol. 82, 303-383.

Pflüger,E. (1901). Die Resorption der Fette vollzieht sich dadurch, dass sie in Wässrige Lösung gebracht werden.
Pflüg.Arch.ges.Physiol. 86, 1-46.

Pflüger,E. (1902a). Fortgesetzte untersuchung über die in Wasserlöslicher form sich Vollziehende Resorption der Fette.
Pflüg.Arch.des.Physiol. 88, 299-338.

Pflüger,E.(1902b). Ueber die bedeutung der seifen für die Resorption der Fette.
Pflüg.Arch.ges.Physiol. 88, 431-452.

Pflüger,E. (1902c). Ueber die Verseifung, welche durch die Galle vermitteltwird, und die Bestimmung von seifen neben Fettsäuren in Gallenmischungen.
Pflüg.Arch.ges.Physiol. 90, 1-32.

Phillipson,A.T.(1947). The Production of fatty acids in the Alimentary Tract of the dog.
J.Exptl.Biol. 23, 346-349.

Phillipson,A.T.(1952a). The passage of digesta from the abomasum of the sheep.
J.Physiol. 116, 84-97.

Phillipson,A.T.(1952b). The fatty acids present in the rumen of lambs fed on a flaked maize ration
Brit.J.Nutr. 6, 190-198.

Phillipson,A.T.(1953). Digestion of cellulose by ruminants.
Biochem.J. 54, iii-iv.

Platt,B.S. (1954). The behaviour of milk in the stomach of the infant rat.
Brit.J.Nutr. 13, Proc.Nutrition Soc. xvii-xviii.

Platt, B.S. (1954). The fate of successive portions of a meal in the rat's stomach.
Brit.J.Nutr. 13, Proc.Nutr.Soc. xvi-xvii.

Popper,H. & Volk,B.W.(1944). Absorption of Vitamin A in the rat intestine.
Fed.Proc. 3, 91.

Poulain,M.A.(1902). De l'action des ganglions lymphatiques sur l'absorption et la resorption des graisses.
Revue Mens.des Maladies de L'Enfance 20, 289-306.

Poynton,F.J. & Paterson,H.(1914). The occurrence of ascites of a non-tuberculous origin in chronic recurrent diarrhoea in childhood.
Lancet I. 1533-1535.

Pratt,T.A. & Frew,H.W.D.(1931). Chylous ascites complicating abdominal lymphadenoma.
Brit.med.J. I. 622-623.

Prenant,A. (1896). Sur la présence d'amas leucocytaires dans l'epithélium pharyngien et oesophagien d'anguis fragilis.
Bibliogr.Anat. I. 21-26.

Prenant,A. (1899). Cellules vibratiles et cellules à plateau.
Bibliogr.Anat. 7, 21-38.

Recklinghausen, (1862). Die Lymphgefässe. Berlin.

Reiser,R. & Bryson,M.J.(1951). Route of Absorption of free fatty acids and Triglycerides from the Intestine.
J.biol.Chem. 189, 87-91.

Reiser,R., Bryson,M.J., Carr,M.J. & Kuiken,K.A.(1952). The intestinal Absorption of Triglycerides.
J.biol.Chem. 194, 131-138.

Reiser,R. & Williams,M.C. (1953). Dihydroxyacetone esters as precursors of Triglycerides during intestinal absorption.
J.biol.Chem. 202, 815-819.

Rekers,P.E., Abels,J.C. & Rhoads,C.P.(1943). Metabolic Studies in Patients with Gastro-Intestinal Cancer.IV. Fat Metabolism, a Method of Study.
J.clin.Invest. 22, 243-248.

Reuter,K. (1901). Zur Frage der Darmresorption.
Anat.Anz. 19, 198-203.

Reuter,K.(1903). Ein Beiträge zur Frage der Darmresorption.
Anat.H. 21, 121-144.

Richter,C.P., Holt,L.E., Barelare,B.(Jr.) & Hawkes,C.D. (1938). Changes in Fat, Carbohydrate and Protein Appetite in Vitamin B. Deficiency.
Amer.J.Physiol. 124, 596-602.

Rindfleisch,E. (1861). In wie fern und auf welche Weise gestattet der Bau der verschiedenen Schleimhaüte den Durchgang von Blutkörperchen und anderen kleinen Theilen und ihre Aufnahme in die Gefässe.
Virchows Arch. 22, 260-285.

Rohmann,F. (1887).
Pflüg.Arch.ges.Physiol. 41, 411.
Cited by Angevine,R.W.(1929) in Excretion by Thiry-Vella Fistulas.
J.Biol.Chem. 82, 559-565.

Rony,H.R., Mortimer,B. & Ivy,A.C.(1932). Fat transport through the lymph system in fasting and Phlorhizin poisoning.
J.biol.Chem. 96, 737-743.

Rony,H.R, Mortimer,B. & Ivy,A.C.(1933). The source of the lipids found in the thoracic duct lymph in fasting. - Endogenous lipid secretion & reabsorption in the bowel.
J.biol.Chem. 102, 161-169.

Rossi,G. (1907). Sull' assorbimento dei saponi e degli acidi grassi.
Arch.Fisiol. 4, 429-492.

Rossi,G. (1908). Della particelare localizzazione nelle cellule dell' epitelio intestinale di sostanze che sciolgono l'acido oleico.
Arch.Fisiol. 5, 381-393.

Rubner,M. (1879). Ueber die Ausnützung einiger Nahrungsmittel im Darmcanale des Menschen.
Z.Biol. 15, 115-202.

Sabin,F.R., Cunningham,R.S., Doan,C.A. & Kindwall,J.A. (1925). The Normal Rhythm of the white blood cells. Johns Hopkins Hosp.Bull. 37, 14-67.

Schäfer,E.A. (1876). Quain's Anatomy, Vol.II. 8th.Ed.p.363.

Schäfer,E.A. (1877). Practical Histology. p.194.London.

Schäfer,E.A. (1884). Ueber die Fettresorption im Dünndarme. Pflüg.Arch.ges.Physiol. 33, 513-514.

Schäfer,E.A. (1885). On the part played by amoeboid cells in the process of intestinal absorption. Internat.Mschr.Anat.und Histologie. 2. 6-29.

Schäfer,E.A. (1912). The small intestine - absorption of fats. In Quain's Anatomy, Vol.II. 11th.Ed. p.541 et seq.

Schmidt,A. (1897a). Experimentelle und klinische Untersuchungen über Functionsprüfung des Darmes. Deutsches Arch.f.Klin.Med.,Leipz.61, 280-545.

Schmidt,A. (1897b). Ueber Schleim im Stuhlgang.
Z.klin.Med. 32, 260-279.

Schönheimer,R. & Behring,H.von (1930). Über die Exkretion gesättigter Sterine.
Hoppe-Seyl.Z. 192, 102-111.

Seckel,H.P.G. & Kato,K. (1938). Development of the diurnal cycle of liver function in nursing rats.
Arch.Path. 25, 347-360.

Settles,E.L. (1920). The effect of high fat diet upon the growth of the lymphoid tissue.
Anat.Rec. 20, 61-93.

Sheehan,H.L. (1939). The staining of Leucocyte granules by Sudan Black B.
J.Path.Bact. 49, 580-581.

Sherlock,S. & Walshe,V. (1946). The use of a portal anastomotic vein for absorption studies in man.
Clin.Sci. 6, 113-123.

Short,A.R. & Bywaters,H.W. (1913). Amino-acids and sugars in rectal feeding.
Brit.med.J. I, 1361-1367.

Shoshkes,M., Geyer,R.P. & Starr, F.J. (1950). Fat Emulsions for Oral Nutrition.
J.Lab.Clin.Med. 35, 968-975.

Sinclair,R.G. (1929). The role of the phospholipids of the intestinal mucosa in fat absorption.
J.biol.Chem. 82, 117-136.

Sinclair,R.G. (1936). Blood phospholipid as a transport mechanism.
J.biol.Chem. 115, 211-220.

Sobel,A.E., Besman,L. & Kramer,B. (1949). Vitamin A Absorption in the Newborn.
Amer.J.Dis.Child. 77, 576-591.

Sperry,W.M. (1926a). Lipid excretion. III. Further studies of the quantitative relations of the fecal lipids.
J.biol.Chem. 68, 357-383.

Sperry,W.M. (1926b). Lipid excretion. IV. A study of the relationship of the bile to the fecal lipids with special reference to certain problems of sterol metabolism. J.biol.Chem. 71, 351-378.

Sperry,W.M. (1929a). Lipid excretion.V. A study of the partition of the fecal lipids with special reference to bacteria. J.biol.Chem. 81, 299-319.

Sperry,W.M. (1929b). Lipid excretion. VII. The partition of fecal lipids in bile fistula dogs. J.biol.Chem. 85, 455-463.

Sperry,W.M. (1932). Lipid excretion. VIII. The lipid content of the intestinal mucosa. J.biol.Chem. 96, 759-768.

Sperry,W.M. & Angevine,R.W. (1932). Lipid excretion. IX. The secretion of lipids into the intestine. J.biol.Chem. 96, 769-786.

Sperry,W.M.& Bloor,W.R. (1924). Fat excretion. 2. The Quantitative relations of the fecal lipids. J.biol.Chem. 60, 261-287.

Stheeman,H.A.(1910). Histologische Untersuchungen über die Beziehungen des Fettes zu den Lymphdrüsen.
Beitr.path.Anat. 48, 170-204.

Stöhr,P. (1884). Ueber die peripheren Lymphdrüsen.
Sitzungsber.der Phys.med.ges.Würzburg,86-94.

Stryker,W.A. (1941).Absorption of liquid petrolatum ("Mineral Oil") from the intestine - a histologic and chemical study.
Arch.Path. 31, 670-692.

Sturgis,C.C. & Bethell,F.H.(1943). Quantitative & Qualitative variations in normal leukocytes.
Physiol.Rev. 23, 279-303.

Tantinø,E. (1935). L'azione della prolungata somministrazione orale dell'slio di vaselina.
Tumori 9, 266-273.

Teichman (1861) (Das Saugader System).
Cited by Watney,H.R. (1876) in The Minute Anatomy of the Alimentary Canal.
Philos.Trans. 166, 451-488.

Thanhoffer,L. (1874). Beiträge zur Fettresorption und Histologischen Struktur der Dünndarmzotten. Pflüg.Arch.ges.Physiol. 8, 391-443.

Thiry,L. (1864). Sitzungsber.Akad.Wiss.Wien.I, 168. Cited by Angevine,R.W.(1929) in Excretion by Thiry-Vella Fistulas. J.biol.Chem. 82, 559-565.

Thorpe,W.V. (1947). Biochemistry for Medical Students. 4th.Ed. p. 417. London:Churchill.

Tidwell,H.C. (1950). Mechanism of fat absorption as evidenced by chylomicrographic studies. J.biol.Chem. 182, 405-414.

Tidwell,H.C. & Nagler,M.E.(1952). Exogenous emulsifiers and fat absorption. Proc.Soc.Exper.Biol.& Med. 81, 12-15.

Tidwell,H.C. & Nagler,M.E.(1953). Effect of emulsifiers on fat absorption in the normal young adult. Gastroenterology 23, 470-476.

Twort,C.C. & Twort,J.M.(1933). The fatty infiltration of the liver of mice resulting from the ingestion of medical liquid paraffin. J.Hyg.,Camb. 33, 396-401.

Twort,J.M. & Twort,C.C.(1932). Changes in the liver of mice, following the ingestion of hydrocarbon oils. Lancet I. 448-449.

Vella,L. (1886). Untersuchungen zur Naturlehre der Menschen und de Tiere, Giessen, 12, 471. Cited by Angevine,R.W.(1929) in J.Biol.Chem. 82, 553-565.

Verzár,F. & Laszt,L.(1935). Nebennierenrinde und Fettresorption. Biochem.Z. 278, 396-400.

Verzár,F. & McDougall,E.J.(1936). Absorption from the Intestine. London: Longmans,Green & Co.

Virchow (1853). Wurz.Verhand 4. Cited by Watney,H.R.(1876) in The Minute Anatomy of the Alimentary Canal. Philos.Trans. 166, 451-488.

Voit,C. & Bauer,J. (1869). Über die Aufsaugung im Dick-und Dünndarm.
Z.Biol. 5, 536-570.

Voit,F. (1892). Beiträge zur Frage der Secretion und Resorption im Dünndarm.
Z.Biol. 29, 325-397.

Volhard,F. (1901). Ueber das Fett~~f~~spaltende ferment des Magens.
Z.klin.Med. 42, 414-429.

Volk,B.W. & Popper,H. (1950). The influence of dispersion upon the absorption of Vitamin A & fat as studied by fluorescence microscopy.
Gastroenterology 14, 549-557.

Watney,H.R. (1876). The minute anatomy of the alimentary canal.
Philos.Trans. 166, 451-488.

Webb,R.L.(1952). The lymphatic system.
Annu.Rev.Physiol. 14, 315-330.

Weber,E.H. (1847). Ueber den Mechanismus der Einsaugung des Speisesaftes.
Arch.Anat.Physiol. 6, 400-402.

Weill,P. (1920). Über die leukocytären Elemente der Darmschleimhaut der Säugetiere.
Arch.mikr.Anat. 93, 1-81.

Weiner,P. (1928). Über Fettablagerung und Fettresorption im Darm.
Z.mikr.-anat.Forsch. 13, 197-268.

Weiner,P. (1932). Über Fettablagerung und Fettresorption II. Die Späten Resorptionsstadien.
Z.mikr.-anat.Forsch. 30, 193-256.

Weiss,O. (1912). Die Resorption des Fettes im Magen.
Pflüg. Arch.ges.Physiol. 144, 540-543.

Whitehead,R.H.(1909). A note on the absorption of fat.
Amer.J.Physiol. 24, 294-296.

Whitehead,R.H.(1910). The absorption of fat stained by Sudan III.
J.biol.Chem. 7, xxvii-xxix.

Whittaker,L.D. & Bargen,J.A.(1937). Observations on the human being following colectomy or colonic exclusion with ileostomy.
Surg.Gynec.Obstet. 64, 849-862.

Wiemer,O. (1884). Ueber den Mechanismus der Fettresorption.
Pflüg.Arch.ges.Physiol. 33, 515-537.

Wilhelmj,C.M.(1952). Physiology of the Digestive System.
Annu.Rev.Physiol. 14, 177-204.

Winiwarter,H.de (1930). Influence du régime albumineux sur le nombre des leucocytes de la muquese intestinale de la souris.
Compt.Rend.Soc.Biol. 104, 121-123.

Winter,I.C. & Crandall,L.A.(1941). The question of the portal absorption of fatty acids.
J.biol.Chem. 140, 97-104.

Wislocki,G.B. & Dempsey,E.W.(1946). Observations on chemical cytology of normal blood & hemopoietic tissues.
Anat.Rec. 96, 249-277.

Wotton,R.M. & Zwemar,R.L.(1939). Studies on direct and visible ingestion of fat by differentiated body cells of the cat.
Anat.Rec. 75, 493-507.

Wuttig,H.(1905). Experimentelle Untersuchungen über Fettaufnahme und Fettablagerung.
Beitr.path.Anat. 37, 378-410.

Yamakawa,S. & Fujinaga,J.(1929). Zur Frage der Resorption des emulgierten Fetts von Dickdarm aus.
Tohoku J.exp.Med. 14, 265-274.

Zawarykin,T.(1883). Ueber die Fettresorption im Dünndarme.
Pflüg.Arch.ges.Physiol. 31, 231-240.

Zawarykin,T. (1885). Einige die Fettresorption im Dünndarme betreffende Bemerkungen.
Pflüg. Arch.ges.Physiol. 35, 145-157.

Ziegler,K.(1921a). Experimentelle untersuchungen über die Resorption von Fremdkörpern in der Bauchhöhle und ihre Pathogenetische Bedeutung für Leberund Milzerkrankungen.
Z. ges.exp.Med. 24, 223-241.

Ziegler,A. (1921b). Über parenterale Resorption und Transport von Neutralfett. Z.ges.exp.Med. 24, 242-261.

Zilversmit,D.B., Chaikoff,I.L., & Entenman,C.(1948). Are phospholipids obligatory participants in fat transport across the intestinal wall? J.biol.Chem. 172, 637-650.

Zimmermann,K.W.(1898). Beiträge zur Kenntniss einiger Drüsen und Epithelien. Arch.mikr.Anat. 52, 552-706.

Zucker,T.F. (1920). Studies in the Absorption of Fats. Proc.Soc.exp.Biol., N.Y. 17, 89-91.