"Well, he finally decided to clean the attic. Almost had the job done, too..."

"...Yeah, until he tried to lift me. It sure put his back out of whack. His doctor's got a real job to do—trying to ease both the pain and the strain."
When stress results in muscle strain and pain

When the normally sedentary person suddenly turns active—cleaning the attic, for instance—the outcome is sometimes a strain or sprain in the back, neck or shoulders.

Fortunately, however, most patients with muscle spasm and pain are highly responsive to therapy with Robaxisal. This rationally based formula provides the well-known relaxant benefits of methocarbamol for strained, tense skeletal muscle plus the dependable analgesic and anti-inflammatory effects of aspirin. Investigators have found methocarbamol a well tolerated agent with "specificity of action." And methocarbamol potentiates the salicylate levels of aspirin so that, in combination, higher salicylate levels are produced than with equivalent doses of aspirin alone. When the Robaxisal combination was administered to a group of 22 patients with painful musculoskeletal disorders, 20 (91 per cent) showed an excellent or good response.

With Robaxisal you can conveniently fulfill the most important objectives in treatment of muscle spasm: relaxation of skeletal muscle, relief of pain, restoration of mobility and normal muscle tone. And when mild anxiety is a factor in the spasm-pain syndrome, consider Robaxisal®-PH.

*In this investigation, 400 mg. methocarbamol was combined with 300 mg. aspirin.


Robaxisal® brings relief for both

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Manuscripts, submitted in duplicate, should be prepared according to recommendations in the Style Manual for Biological Journals, 2nd ed., published in 1964 by the American Institute of Biological Sciences, 2000 P Street, N.W., Washington, D. C. 20036.

Subscription rates in the USA and Canada: 1 year, $4; 2 years, $7; 3 years, $9. All other countries: 1 year, $5; 2 years, $8; 3 years, $10. Interns, residents, and students: 1 year, $2.

Correspondence: MEDICAL COLLEGE OF VIRGINIA QUARTERLY, Medical College of Virginia, Richmond, Va. 23219. Phone: 703/770-4027.

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Pancreatitis

FRANZ J. INGELFINGER*

The New England Journal of Medicine, Boston, Massachusetts 02115

I am, of course, most grateful to everyone responsible for arranging this—the 39th McGuire Lecture Series. It is a series distinguished by tradition: few medical lecture-ships can point to a continuity of nearly 40 years, and few can boast of the three M's of American medicine—Mayo, Mann and Minot. Incidentally, going over the list of previous lectures, I could not help noticing that I am the eighth Bostonian in this series, which puts us well ahead of our nearest competitor, a clinic in a state with a name that also happens to begin with M.

My pleasure at being invited to give this Stuart McGuire Lecture has naturally been doubled—and I am speaking in logarithmic terms—by having on the same program 11 men who had, let us say, some experience in our gastrointestinal section in Boston. Dr. Farrar, Dr. Caravati and others, in arranging this, were doubtlessly motivated by generosity but also probably felt a bit sorry for the old man who has entered what some regard as the retirement of an editorship.

The subject of my lecture requires better definition: I shall talk about pancreatitis and, chiefly, about the diagnosis of the recurrent variety of pancreatitis. How can we recognize the pain of this disease? This pain does tend to be in the upper abdomen, more often on the left than the right, and radiates to the back in about a quarter of the cases. It has, however, no characteristic quality, and the degree of the pain may range from the relatively painless to the excruciating. Most often, its severity is between these two extremes.

In an essay on pain published in 1930, Dr. Stuart McGuire wrote as follows:

In considering the significance of pain which occurs in patients who are suddenly taken with belly-ache, the vital question to decide is whether it is caused by an acute intra-abdominal disease such as appendicitis, cholecystitis, perforating ulcer or intestinal obstruction—conditions which demand prompt operative intervention—or whether it is reflex from extra-abdominal diseases such as tabes, angina, pneumonia, lead poisoning, rheumatic conditions or diseases of the upper urinary tract—conditions which call for medical rather than surgical treatment.

Note that, among the common causes of abdominal pain listed, pancreatitis does not appear. He certainly knew about pancreatitis, but my guess is that Dr. McGuire considered it an uncommon ailment. The Index Medicus in those years usually had less than 50 entries annually relating to pancreatitis, and chronic or recurring pancreatitis was hardly mentioned. In contrast, the 1966 Index Medicus contained over 350 entries, and as one scans the list, the preponderance of reports originating either from the United States or France is striking. Articles from Israel, on the other hand, are conspicuously absent. Indeed, when an experienced surgeon from Israel was recently shown one of Boston's many patients with chronic alcoholic pancreatitis, he doubted the diagnosis. Why? Because, he said, it was such a very, very rare disease. He had never seen a case.

Alcohol the Villain

What do the United States and France have that Israel does not? Enthusiastic consumption of alcohol. The evidence is very suggestive that the prevalence of pancreatitis and the consumption of alcohol show parallel trends in various areas of the world, even as cirrhosis and alcohol consumption show parallel trends. The beer-drinking Bantu of South Africa, for example, not only has much cirrhosis; he also has much chronic pancreatitis.

Dr. Gerald Klatskin of Yale likes to show a graph plotting deaths from cirrhosis against years. After the enactment of prohibition, the rate fell sharply from about 12 to 7 per 100,000, but after drinking became legal again, the death rate from cirrhosis steadily rose to a level of about 11 (Klatskin, 1961). Adequate figures for plotting the incidence of pancreatitis over the years are not available, but my guess is that this incidence has steadily gone up since the repeal of prohibition.

The relation of pancreatitis to alcohol consumption is not only of epidemiologic interest but also has a bearing on our subject: abdominal pains in a patient who drinks alcohol should make one think of pancreatitis. You may regard this as too obvious. But the following happens again and again in Boston, and I suspect elsewhere as well. A patient complains that he has vomited and has abdominal...
pain coming on a few hours after some reasonably heavy drinking. The vomitus may or may not contain blood, but the chances are nearly 100% that a diagnosis of alcoholic gastritis will be made. The same story may repeat itself a few times until someone happens to take a serum amylase which, if elevated, helps establish the correct diagnosis. Serum amylase, however, is not always elevated under such conditions, and the correct diagnosis may never be suspected until an abdominal x-ray one day reveals calcification in the pancreas.

Not all abdominal pains in patients who have been drinking are caused by pancreatitis, but the possibility is a real one. That alcoholic gastritis is responsible for abdominal pains appearing in the drinking patient, however, is not a good possibility for the simple reason that the existence of such an entity is controversial and, in any case, its symptomatology quite obscure. No one has yet shown that the histologic appearance of the stomach in the patient who is drinking is different from that found in a control population, or that the gastroscopic appearance of the stomach in a patient vomiting because of alcoholism looks different from that of a patient vomiting for some other reason.

It is, of course, silly to talk about drinking without defining its degree.

In cirrhosis of the liver before the liver clinically manifests has to be hou. Pancreatitis, hand, does not ; a background his drinking, and alt may be he be intermittent. P get their first at pancreatitis, usua been eating well, It is striking that alcoholic pancrea cirrhosis of the l monly seen in . Possibly the diffe individual sucse also possible that it first hits, hits t nutritionally in g.

Table 1 shows alcohol so insister delicious beverage at least half the titis that you wil case of recurrent percentage is pr other words, awa sociation is cruci tial diagnosis of pain.

Other Background

When Dr. McC abdominal pain, considered the primary cause of pancreatitis, responsible for some 80% of attacks. Their contribution to the etiology of pancreatitis, however, is rapidly decreasing, partly on a relative basis because of the increasing frequency of alcoholic pancreatitis, and partly because symptomatic gallstones are so promptly removed these days that pancreatitis caused by neglected biliary tract disease is becoming a rarity.

Whether peptic ulcer really initiates pancreatitis by eroding into the pancreas, with subsequent spread to involve more of the organ, is controversial. In my opinion it is an uncommon but not rare antecedent of pancreatitis.

Trauma clearly appears to be an antecedent of pancreatitis, sometimes on the table and sometimes off. The “on the table” variety is usually precipitated by vigorous manipulation of the upper abdominal cavity during the course of gastro-duodenal or biliary tract surgery. The “off the table” kind is caused by blunt and non-penetrating trauma to the belly such as occurs when the body is thrown against a steering wheel, or when someone dives into a swimming pool, not realizing that the water has been emptied out of it. Since this phenomenon admittedly is only apt to happen under certain conditions, the dilemma is posed whether the dive or the pre-dive alcohol is responsible. As a matter of fact, with the exception of gallstones, the same question applies to most of the possible background conditions listed. Many a patient with pancreatitis, for example, has both peptic ulcer and a tendency to drink alcohol in excess.

Malnutrition is often invoked as a background condition responsible for pancreatitis, because the pancreas is histologically altered in kwashiorkor and because the methionine antagonist, ethionine, produces a chronic pancreatic reaction in experimental animals. Direct evidence, however, is lacking. Many

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**TABLE 1**

<table>
<thead>
<tr>
<th>Associates of Pancreatitis</th>
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<tr>
<td><strong>Causal (%)</strong></td>
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<tr>
<td>50</td>
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<td>25</td>
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<td>1</td>
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<td>0</td>
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*If “none”: chances of pancreatitis < 10%*
natives of Uganda, to be sure, have chronic pancreatitis with calcification of the organ, but Ugandans appear to be as intemperate as Americans and French, and, hence, the problem is again one of selecting the real culprit from at least two candidates.

Another disorder apt to affect malnourished populations is parasitism. The parasite most apt to cause pancreatitis is *Ascaris lumbricoides*, which crawls into the common duct to obstruct it. In our adult US population, especially in people of higher socio-economic status, ductal pancreatic obstruction by this mechanism is almost unheard of. On the other hand, if a population of children in an impoverished tropical country is under consideration, parasitism turns out to be the principal cause of pancreatitis. These facts underscore the difficulty of arriving at universally applicable percentage figures for the frequency of various etiologies of pancreatitis. The figures shown in Table 1 are approximations of the crudest type.

Metabolic diseases other than diabetes have been well publicized as causes of pancreatitis, and the recent literature incriminates a number of drugs that are also suspect (Table 2). Although fascinating because of their diagnostic and etiologic implications, metabolic disorders and drug reactions account, at least in my opinion, for only a small proportion of all cases of pancreatitis. When hyperlipemia is associated with pancreatitis, either condition may be cart or horse. Patients with essential hyperlipemia are, for unknown reasons, susceptible to attacks of pancreatitis. Conversely, hyperlipemia in other instances is the consequence of repeated attacks of pancreatitis, often alcoholically induced. Hyperlipemia, thus, may be found constantly in about 5% of patients with chronic recurrent pancreatitis, and, when present, may in some mysterious way suppress the increases in serum amylase and lipase usually expected when pancreatic inflammation is active (Greenberger et al., 1966).

The association of pancreatitis with hyperparathyroidism also tends to be emphasized, but the absolute number of such cases is small, except at the Massachusetts General Hospital, a magnet for patients with parathyroid dysfunction. At this famous institution, indeed, the significance of blood calcium levels is uncertain, for obviously the hypercalcemia of hyperparathyroidism and the hypocalcemia of severe pancreatitis may cancel each other. Finally, pancreatitis appears to be associated in certain families with amino-aciduria, particularly of lysine and cysteine. In a number of these patients, however, alcoholic intake is heavy, and, again, the real criminal is hard to identify. The situation resembles that of hemochromatosis. Many think that hemochromatosis is a genetically transmitted disorder, but since so many hemochromatotics drink, one almost has to assume that a penchant for drinking alcohol is also genetically transmitted.

In about 10% of the cases, pancreatitis affects a patient who has no recognizable background condition.

**Complications of Pancreatitis**

Many of the disorders listed in Table 1 may be regarded as the consequences (right-hand column of percentage figures) rather than the causes of pancreatitis. Is it really true that 1% of patients are alcoholic because they have pancreatitis? I doubt it, but, at one time, when alcoholism was not a socially acceptable diagnosis, it was suggested that some people took up drinking because their abdominal pain was so bad. It reminds me of one of our patients who always made the two-glass test when he got one of his attacks of pancreatitis. With the onset of pain, he would promptly repair to the local bar and take two shots of whiskey. If this made the pain disappear, well and good—he could drink more. If it did not make the pain disappear, he had to keep on drinking so that he might forget the pain.

Can chronic pancreatitis cause gallstones? There is considerable evidence that one of the background conditions necessary for precipitation of biliary sediment is stasis, and, hence, chronic pancreatitis, to the extent that it obstructs the biliary passages, may lead to the production of gallstones. The 5% is obviously a very rough guess.

The precipitation of pancreatitis by peptic ulcer is, as I have indicated, debatable. On the other hand, there is very good evidence to suggest that peptic ulcer may result from chronic pancreatic insufficiency. The patient who puts out pancreatic juice grossly deficient in bicarbonate because of pancreatic insufficiency obviously cannot neutralize gastric contents in a normal fashion, and, hence, his duodenal contents may be abnormally acid. In addition, it has been shown that the absence of normal pancreatic juice in the duodenum itself stimulates gastric secretion. Thus, both enhanced gastric acid secretion and decreased neutralization of the duodenum join forces to make the patient with chronic pancreatic disease susceptible to peptic ulcer.

Try as I might, I could not figure out how pancreatitis would lead to increased trauma, unless one wanted

<table>
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<th>TABLE 2</th>
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<td><strong>Conditions and Drugs Suspected of Causing Pancreatitis</strong></td>
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<tr>
<td><strong>Metabolic Disorders:</strong></td>
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<tr>
<td><strong>Drugs:</strong></td>
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to argue that the driver seized by the belly pain of pancreatitis will smash into somebody else. If pancreatitis is so severe that pain and vomiting interfere with eating, or if digestion is impaired by pancreatic insufficiency, malnutrition obviously ensues. Similarly, diabetes is a frequent concomitant and result of chronic pancreatitis. Many patients with chronic pancreatitis use narcotics and, after a while, it is hard to tell whether they are addicted or really need the medication. In any case, abuse of narcotic drugs is certainly the result in some patients with pancreatitis. To the extent opiates and meperidine cause further spastic obstruction at the sphincter of Oddi, the use of these drugs may in turn aggravate the original disease.

The percentage figures in both columns of Table 1 are not only imprecise; they are also grossly affected by a great deal of overlap. Many patients with pancreatitis have both diabetes and malnutrition. If the percentages indicating prevalence on both sides are added, their total is 180%, as might be expected, since one patient with pancreatitis often has several associated conditions.

The diagnostic importance of Table 1 is that there are relatively few patients with pancreatitis who have no associated conditions whatever. When the prevalence of conditions associated, for one reason or another, with pancreatitis is taken into account, one may logically conclude that less than 10% of patients with pancreatitis have pancreatitis all by itself, without any of the conditions listed in Table 1. Put another way, this means that, if one sees a patient with upper abdominal pains of unexplained nature and he has none of these conditions associated with pancreatitis, the chances are less than 10% that he is suffering from pancreatitis. The converse is, of course, not true. Many patients with abdominal pain have alcoholism, gallstones, or diabetes and are not necessarily suffering from pancreatitis. The list in Table 1 presents criteria that are negatively important: their absence militates against the diagnosis of pancreatitis.

Extra-Pancreatic Manifestations

Recognition of pancreatic pain is not only aided by an awareness of those conditions that are associated historically with pancreatitis. Equally helpful are extra-pancreatic phenomena often appearing during the acute episode (Table 3). The presence of such phenomena is related to the severity of the attack. The more drastic the attack, the more likely that other systems will be involved. Particularly frequent are radiologic signs of pleural or pulmonary changes; even in the moderate cases, one may expect them about a third of the time. The cardiovascular reactions to severe pancreatitis are well-known. Less emphasized are some of the unusual manifestations of fat necrosis such as aching in the bones, possibly related to necrosis of marrow fat, and, very rarely, subcutaneous nodules reminiscent of Weber-Christian syndrome. One of the explanations for the tetany that occurs a few days after an attack of severe pancreatitis is liberation of free fatty acids which then, in turn, bind calcium to form soaps.

Central nervous system disorders associated with pancreatitis are sometimes striking. In my experience they have been limited to those patients who have alcoholic pancreatitis and, consequently, are particularly suscetible, because of chronic cerebral damage, to the vascular and blood flow derangements that attend pancreatitis. In a few cases, in addition, large doses of atropine or of similar agents given for therapy aggravate the delirium.

Bleeding phenomena, although unusual, do take place, and one of the most striking is the appearance of a bluish-black discoloration on the flanks, the so-called Grey-Turner sign. How do you spell it? Is it with an e or with an a? Whichever it is, is it hyphenated or not? Is it one person or two persons? Man or woman? I ask these questions because journals and textbooks seem to be quite inconsistent. One textbook spells it with an a; another one seems to hyphenate it in some places and not in others. I emphasize this type of niggling because it epitomizes the editor's life, and only editorial experience reveals the intricacies of truth. It was just one person. There was no hyphen ever between the Grey and the Turner. However, it is apparently correct usage that if a man's two names are used as an adjective, then the hyphen is properly placed between them. Grey Turner, the man, is not hyphenated, but Grey-Turner sign is.

The Enzymatic Attack

What is responsible for all these phenomena, as well as for the major presenting complaint—namely, that of pain? Presumably it is the digestive enzymes of the pancreas.

<table>
<thead>
<tr>
<th>TABLE 3</th>
<th>Pancreatitis: Involvement of Other Systems</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pleura-Lungs</td>
<td>effusions, infiltrations</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>shock, hypovolemia</td>
</tr>
<tr>
<td>Fat Depots</td>
<td>necrosis (deep, bone, subcutaneous) (tetany)</td>
</tr>
<tr>
<td>CNS</td>
<td>delirium</td>
</tr>
<tr>
<td>Clotting</td>
<td>hemorrhages</td>
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</tbody>
</table>

<table>
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<tr>
<th>TABLE 4</th>
<th>Pancreatic Enzymes</th>
</tr>
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<tbody>
<tr>
<td>Trypsin</td>
<td>Lipase</td>
</tr>
<tr>
<td>Chymotrypsin</td>
<td>Other Esterases</td>
</tr>
<tr>
<td>Carboxypeptidase</td>
<td></td>
</tr>
<tr>
<td>Phospholipase A (Lecithinase, Phosphatidase)</td>
<td></td>
</tr>
<tr>
<td>Elastase</td>
<td>Amylase</td>
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<tr>
<td>Collagenase</td>
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F. J. INGEFINGER
attacking the patient's own tissues. Proteolytic and lipolytic ferments, listed in Table 4, are disrupting the basic constituents of tissue. Interestingly enough, amylase, the enzyme which we most depend upon for diagnosis, presumably does little damage when it accumulates in the bloodstream.

Since trypsin and its congeners are so implicated in initiating and perpetuating both the local and distant deleterious effects of pancreatitis, it would seem reasonable to measure the activity of these proteolytic enzymes in the blood and other body fluids, not only for the purpose of diagnosis, but also to assess the severity of the process—much as we use transaminases in the diagnosis of acute liver disease. A priori tests of proteolytic activity should be easy to devise. Trypsin was one of the first mammalian digestive enzymes to be isolated and crystallized, practically 40 years ago. The brilliant research of Neurath and others, furthermore, has defined in a nearly complete fashion both the chemical and steric structure of trypsin and chymotrypsin, and of their inactive precursors, trypsinogen and chymotrypsinogen (Neurath, 1964). Chymotrypsinogen, the longer chain of the two, consists of 246 amino acids in a curlicue chain interlinked at various points by sulfide bridges. When the terminal 15 amino acids are split off, the enzyme becomes active, i.e., it is chymotrypsin, presumably because removal of the end chain permits steric reorganization with the creation of active enzymatic sites. The chain of trypsinogen in general looks about the same except that it contains 229 rather than 246 amino acids.

Though trypsin and chymotrypsin look alike, they are highly fastidious in selecting where they attack the proteins they digest. Amino acids making up proteins are joined by bridges that form between amide and carboxyl terminals of amino acids. When trypsin splits the peptide linkages, it attacks only those that are next to an amino acid with a positively charged side group, such as arginine. Chymotrypsin splits only bridges that are next to amino acids with a six-carbon ring, such as tyrosine.

So what more is needed? The perpetrators of pancreatitis, trypsin and chymotrypsin, are chemically recognized, and specific substrates are available to catch them. Indeed, this substrate specificity has been exploited by the synthesis of relatively simple materials and by the use of these, rather than of complex proteins, for substrates. For example, an amide of a derivative of arginine can be made, such as benzoyl argininamide. If this is exposed to trypsin, ammonia is split off, and this ammonia can be measured to obtain an expression of trypsin activity. This principle was first used in the 1930's but was revived about ten years ago as a means of measuring trypsin activity in the blood. More recent modifications have improved the procedure technically. Trypsin and chymotrypsin do not care about the ends of the substrate molecule. If an ester is formed by combining an arginine derivative with methyl or ethyl alcohol, trypsic activity can be measured by titrating the acidity liberated by digestion of the ester. If a tyrosine derivative is used, chymotrypsinogen can be similarly measured.

Problems of Testing Proteolysis

The method does not appear to yield reliable and consistent results when proteolytic activity in the blood is assayed. There are two major reasons for this. In the first place, though trypsin is fastidious, other substances are similarly fastidious and attack the same amino acid linkage. Thus, thrombin, plasmin or fibrinolysin, the first component of complement, and a serum esterase split ammonia from a substrate such as benzoyl argininamide. Trypsin is specific in that it attacks only a certain type of peptide link-age, but the substrate is not specific for trypsin.

The second difficulty is that the body appears to react to proteolytic activity as nature reacts to a vacuum. Whenever proteolytic activity starts to appear in excess, inhibitory substances appear with equal rapidity in an effort to control autodigestion. Some of the very products of trypsin digestion may in themselves be inhibitory. But even in the absence of excess proteolytic activity, body tissues and fluids are well supplied with anti-trypsin substances. Indeed, pancreatic juice in its native stage contains an inhibitor of proteolytic activity, and it may be that enhancement of trypsin digestion is at times attributable less to increased trypsin than to decreased concentration of inhibitor.

The difficulty of recognizing pancreatitis in its moderate and early stages has encouraged a number of ingenious diagnostic approaches. Water, bicarbonate and enzyme output by the pancreas, stimulated by secretin and pancreozymin, can be measured with reasonable satisfaction in the intact human subject. Though direct x-ray of the pancreas is still a goal to be achieved, the organ can be outlined by scanning after giving selenium-75-mer-thionine. Subselective angiography may at times detail the small branches of the arterial system supplying the pancreas. All these methods are highly successful in the advanced cases of pancreatitis, but in such cases, simpler means suffice to make the diagnosis. The usefulness of angiographic and isotopic methods in diagnosing early or diffuse pancreatitis is still circumscribed, and I doubt that they will improve markedly in this respect within the next ten years.

The Amylase Test

Our diagnostic skill must thus rest, as it has rested, on measuring the pancreatic enzyme which apparently does little harm to the body, namely, serum amylase. It
has been shown that under certain conditions the measurement of urinary amylase output—and I emphasize output per unit time, not merely amylase concentration in the urine in a spot sample—may be increased when serum amylases are normal. One cannot say categorically whether measurement of serum or urinary amylase output is better, but if a patient is seen with abdominal pains, and the overall clinical picture includes one or more of the conditions so frequently associated with pancreatitis, serial tests of both serum amylase concentration and of urinary amylase output before, immediately after, and days after the attack are the most successful laboratory means available to us, at present, for recognizing pancreatitis (Gambill and Mason, 1963).

Methods for measuring amylase in body fluids are, in addition, becoming more discriminatory. By appropriate electrophoretic and chromatographic techniques, amylases of salivary and pancreatic origin may be distinguishable both in the urine and in the serum. Thus, a recent English article has indicated that decreased output of urinary amylase may be found in patients with chronic pancreatitis, provided salivary amylase is separated from pancreatic amylase (Aw, Hobbs, and Wootan, 1967). The total urinary amylase in such patients, the authors claim, may be normal, but a decreased proportion contributed by the pancreas may be masked by a corresponding increase in salivary amylase.

Other studies, such as those which have appeared recently in The New England Journal of Medicine (Berk et al., 1967), indicate that serum amylase, as well, may be separated into several components. Just how useful this will be in the recognition of most pancreatic disease is not yet certain, but it appears likely that the source of elevated serum amylase—whether from pancreas, salivary glands or, perhaps, even liver—will be identifiable.

Pending the development of sophisticated techniques that are less cumbersome and more reliable, the diagnosis of pancreatitis will continue to depend on amylase determinations, especially those carried out serially.

References


Cirrhosis: What Is It?*

CHARLES M. CARAVATI
Department of Medicine, Medical College of Virginia
Richmond 23219

"For the King of Babylon stood at the parting of the way, at the head of the two ways, to use divination: he made his arrows bright, he consulted with images, he looked in the liver." Ezekiel 21.
The liver has been looked upon by primitive man as the probable source of health, disease and even of evil spirits, and in 304 B.C. Erasistratus recognized that there was an association between ascites and liver disease. In 1689 John Browne described it as hardening of the liver, and Bailly noted its relation to alcohol in 1793. Then, in 1819, Laennec called it cirrhosis after the Greek word kiros, meaning orange or yellow. Of interest, I believe, is a picture found in Life magazine about ten years ago. It is of a tombstone dated 1790 in a New Hampshire graveyard, the tombstone of a man from whose peritoneal cavity was drawn 2,385 pounds of water. Unquestionably he had cirrhosis.

Definition of Cirrhosis

A proper definition of cirrhosis is difficult, but for this discussion I think the following probably serves our purpose: It is a chronic liver disease characterized by destruction of cells, by the formation of new tissue—particularly connective—and by diffuse regeneration. No effort will be made to offer a classification, because one cannot be found which satisfies the criteria we would like to discuss.

Distribution of Cirrhosis

I would now like to talk about the extent and the implications of the disease itself. The first thing I think we ought to note is that cirrhosis is global—very widespread. About a third of a million people die every year from cirrhosis of the liver; it is the eighth cause of death, and in the age group from 45 to 64, it is surpassed only by cardiovascular and neoplastic disease. It has a very interesting distribution. For instance, in France there are 26 deaths per hundred thousand population; but in England, just across the Channel, there are only two per hundred thousand. In Virginia there are 7.7 deaths per hundred thousand; in New York there are 19. We reviewed the vital statistics of Virginia for five years, 1960-1964, and there were 1,545 deaths, about equally divided between whites and non-whites, with males predominating, and with the average age being from 48 to 50. The records at MCV from 1960-1963 showed 94 cases diagnosed as cirrhosis, but could confirm only 44.

In reviewing the problem of cirrhosis in any area, one is impressed with its probable association with alcohol. By extrapolation, it is accurate to state that more than 50% of cases of cirrhosis are associated with excessive consumption of alcohol. In the Virginia Vital Statistics 33% of the death certificates indicated that alcohol was a probable factor. The Mayo Clinic report of 1950 on a five-year study indicated there was an alcoholic history in 64% of their patients. In Los Angeles in 1953, of 16,000

* Presented at the Thirty-Ninth Annual McGuire Lecture Series, November 9-10, 1967, Medical College of Virginia, Richmond.
autopsies there were 782 cases of cirrhosis, and 78% of these were reported as heavy drinkers. A report from Minneapolis over 20 years ago reported that only 25% of their cirrhotics were associated with alcohol, but I call your attention to the fact that there were only 100 cases studied. It is important to consider that only one alcoholic in 12 develops cirrhosis. What happens to the other 11? Why do they not develop cirrhosis? At this time the answer is not available.

**Etiology of Cirrhosis**

Actually this chronic liver disease probably starts as an acute insult to the liver, to the hepatocyte itself, and to the mesenchymal cells; and then a reaction develops, probably associated with ischemia. The pathological states that are most responsible for this injury are steatosis, necrosis, cholestasis, and hypoxia. In chronic liver disease these processes are perpetuated by persistence of an original offender such as piecemeal necrosis, which is probably the most important factor in the production of cirrhosis. In the proximity of these dead cells, there are immunoglobulin-containing cells—mesenchymal cells—which may assist in the perpetuation of this reaction. A hypersensitivity reaction may be an important factor in the continuation of the inflammatory reaction, but no circulating antibodies to the hepatocyte have been demonstrated, although antibodies to nuclear DNA, as well as to proliferating bile ducts, have been demonstrated. Disturbances in the hepatic circulation must be an important factor in the perpetuation of cirrhosis. These may be most profound. The most striking change, which can be demonstrated clearly by utilizing injection techniques, consists of marked distortion of the hepatic vasculature with pronounced disturbance of the venous outflow tract and post-sinusoidal obstruction.

**Hepatitis**

There are certain clinical disorders that may be etiological factors in liver disease. Let us consider some of these. How big a factor is hepatitis? It is very difficult to document that a patient with primary viral hepatitis develops cirrhosis. He probably does, but actually one has to have biopsy studies over a period of time to prove this. The same thing may be said about ethanol, malnutrition, and others. Schaefer et al., in March of 1967, reported on five individuals with viral hepatitis on whom they performed punch biopsies; these individuals presented a characteristic picture of acute hepatitis with necrosis, inflammation, distortion, and many different types of cells. In biopsies taken some months after the onset, they found fairly normal liver cells without radial pattern and widened scarring with many lymphoid cells, which is a characteristic picture of post-necrotic cirrhosis. In this series, quiescent cirrhosis was found in three of these individuals at the end of 18 months, and all five probably had viral hepatitis. There are now other histological reports available showing progression from viral hepatitis to definite cirrhosis. However, Franken et al. (1967) reexamined 154 patients some years after they had had clinical hepatitis. Thirty-three showed some abnormal biochemical changes, but only three were found to have cirrhosis, and they were alcoholics.

**Malnutrition**

What is the role of malnutrition? Malnutrition's role is not clearly understood. It is thought now that it plays a much smaller role than formerly believed (Jolliffe and Jelinek, 1941). There are few nutritional states in which cirrhosis is common; one of these is chronic ulcerative colitis, in which liver pathology occurs and cirrhosis may develop (Palmer et al., 1964). There are other nutritional disorders in which people do not develop cirrhosis. For instance, in the patient with kwashiorkor, a disease caused by protein deficiency, why is it that the patient does not
develop cirrhosis (Scrimshaw et al., 1959)? A marked fatty liver is very common in this disorder, but it never develops cirrhosis. It is not known why, but it is clear that some fatty livers may be followed by typical "nutritional" cirrhosis, whereas others may not.

Stasis

Stasis, especially that caused by extrahepatic obstruction resulting from benign disorders such as common duct stones, causes cholangitis. This, in turn, may result in cirrhosis of the biliary type, but biliary cirrhosis may be found in the absence of extrabiliary obstruction.

Alcohol

Now what about alcohol? What does alcohol do to the liver? Does it produce a toxic or a metabolic effect? Alcohol can produce a definite direct effect on the hepatic parenchyma. It is not necessary, also, to have malnutrition or the lack of any substance, for alcohol per se is toxic to liver cells. Alcohol may cause a type of hepatitis with necrosis, inflammation, cholestasis, deposition of hyaline; and all these pathological abnormalities may be factors that result in cirrhosis. The steatosis associated with alcoholism may predispose the liver to cirrhosis; at least fatty changes often precede the deposition of fibrous tissue. Does steatosis really cause cirrhosis, and, if so, how does it do it? The work of Leevy (1962) suggests that the fatty liver seen in alcoholics often progresses to cirrhosis, and fat per se may be an important etiological factor in the production of cirrhosis. Hartroft and Ridout (1954) are in agreement with this view. However, some authors, such as Popper, Szanto, and Elias (1955), doubt the role of steatosis as an etiologic agent in cirrhosis but feel strongly that the necrosis of cells produced by alcohol initiates the changes which lead to cirrhosis.

Drugs

While drugs and chemical agents frequently cause liver injury and even death, their use seldom ends in cirrhosis. This is difficult to explain, because the original injury may be indistinguishable from viral hepatitis.

Pathophysiological Disturbances

When cirrhosis occurs, there are multi-system pathophysiological disturbances, which at times are very pronounced. We will discuss a few of the important extrahepatic changes occurring in individuals with cirrhosis. In Figure 1 the chief hepatic alterations are listed within the circle; the organ systems which may be functionally and structurally affected by the liver pathology are indicated outside the circle.

Changes in skin and musculoskeletal system

TABLE 1

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<th>Changes in Skin and Musculoskeletal System</th>
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<td>Icterus</td>
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<td>Pellagra-Like Changes</td>
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<td>Palmar Erythema</td>
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<td>Dilated Veins</td>
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<td>Spider Nevi</td>
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First, let us consider the skin and musculoskeletal system (Table 1). Beginning with the fingers, there is often clubbing of the nails, the so-called Terry nails or the blue nails of Wilson's disease (Kleeburg, 1954; Terry, 1954; Morey, 1955). There is often marked muscle wasting and the characteristic alopecia, as well as Dupuytren's contracture, which Wolfe, Summerskill, and Davidson (1956) state occurs in two-thirds of the people with cirrhosis but, in our experience, is not seen as frequently. Even though Hijams van den Bergh in 1901 described pulmonary osteoarthropathy in a patient with cirrhosis and cyanosis, little emphasis has been placed on it. Recently, however, we have seen several such patients, and each showed low arterial oxygen saturation, which was first reported by Snell (1935). Hansoti and Shah in 1966 reported that one out of seven cases of cirrhosis seen by them in India showed osteoarthropathy. Figure 2 shows a clear x-ray picture of subperiosteal thickening of the tibia characteristic of osteoarthropathy in an individual who has severe cirrhosis. Such osteal changes may be seen quite frequently, if radiological examination of the bones is performed on cirrhotics.

The spider nevus, which is like a coiled end artery raised on the skin with a central pulsating hub from which many divisions of the vessels divide, is actually a vein-like vessel but contains arterial blood. There may be a pressure ranging from 60 to 100 mm Hg in these "spiders." According to Bean (1945), "spiders" can be seen in about 60% of cirrhotics. Why do these angiomas occur in portions of the body supplied by the superior caval vessels? Occasionally they occur in other areas such as the legs, but very rarely, and almost as rarely on the forearms or hands.

Changes in circulatory system

The cardiovascular changes are among the most striking seen in cirrhosis. Some of the more common are listed below.

1. Gross distortion of hepatic vasculature.
2. Portal hypertension.
3. Venous anastomoses.
5. Tachycardia.
6. Increased aortic blood flow.
7. Low arterial oxygen saturation.
8. Increased cardiac output.
9. Short circulation time.
10. Low peripheral vascular resistance.
11. Reduced arterial hypertension with varices.

A hyperdynamic cardiovascular system is manifested by many changes. Many of these are due to multiple A-V shunts that occur in the lungs and pleurae, as well as in the liver, with venous admixture and arterial unsaturation (Hecker and Sherlock, 1956). Heart failure may occur, particularly after surgical shunts.

Schwartz (1967) has recently shown that arterial hypertension is rare in cirrhosis, unless it is associated with renal disease. But if those patients who have renal disease and also have arterial hypertension and cirrhosis develop varices, then the arterial blood pressure becomes normal. If one does an end-to-side shunt on those individuals, the blood pressure goes back again. What does this mean? Is there some substance inhibitory to the hepatic angiotensinase that is diverted from the liver in patients with varices?

Changes in hematopoietic system

The hematological changes occurring in the cirrhotic are many (Table 2). There is frequently a quantitative diminution in platelets, as well as changes in platelet aggregation (Thomas, Ream, and Stuart, 1967), and, at times, thrombasthenia (Mandel and Lazerson, 1961). Further, there may be significant abnormalities in the red and white cell in the presence of anemia and leukopenia or even leukocytosis. There may be folic acid deficiency due to faulty storage (Herbert, Zalusky, and Davidson, 1963), as well as production defects such as a deficit in fibrinogen and prothrombin (Ratnoff, 1963). Production of abnormal complexes, such as the macroglobulins—notably the 7-S macroglobulin—is not infrequent, and recently deficiency in immunoglobulin A has been reported (Wilson et al., 1968). Hemolysis is not uncommon and may be related to the presence of acanthocytes (Douglass, McCall, and Frenkel, 1968). There is quite frequently an excess of fibrinolysins, according to Ratnoff (1963).

Changes in renal system

One of the most important functional derangements is the renal change, the so-called hepato-renal syndrome. Many patients who have advanced cirrhosis die in hepatic failure without any significant disease of the kidney. While specific renal abnormalities which cause renal failure are occasionally demonstrated (Laube, Norris, and Robbins, 1967), they are quite rare. A study in Boston by Garceau and Chalmers (1962) of 253 cases of patients with varices who had died revealed that renal shutdown preceded death in 11% of the cases.

The pathophysiology of the hepato-renal syndrome is still unclear, though reduced glomerular filtration may be one of the mechanisms responsible for the renal failure. There is significant reduction in maximum urine concentration and the rate of solute-free water re-absorption in cirrhosis. This is reversible if hepatic compensation improves (Vaamonde et al., 1967).

Changes in endocrine system

The endocrine features shown below are but a few encountered.

1. Gynecomastia.
2. Hyperestrogenism.
3. Hyperaldosteronism.
4. Amenorrhea.
5. Gonadal atrophy.

Gynecomastia may be marked, and such breast changes, as well as testicular atrophy, are thought to be due to the inability of the liver to conjugate estrogen (Pincus et al., 1951; Morrione, 1944). The most important feature in the management of patients is the increase in serum aldosterone. If one gives 500 mg of aldosterone to a patient with ascites, he will excrete about 15% of this mass. If the same load is given to a normal

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Fig. 2—Radiological appearance of the periosteum showing marked thickening seen frequently in cirrhosis.
individual, he will excrete about 5% of aldosterone. This indicates that the liver is not degrading the aldosterone delivered to it, and, therefore, more of it is going to the kidney. As a consequence, there is more sodium retention. Amenorrhea was reported by Armas-Cruz (1951) in 39 of 66 females, and testicular atrophy was found by Morrione in 90% of males under the age of 50 (1944).

Changes in nervous system

Below are some of the central nervous abnormalities that may be seen in advanced cirrhosis.

1. Encephalopathy.
2. Asterixis.
3. Variable reflexes.
4. Coma.
5. Convulsions.
6. Hemiplegia.
7. EEG.
8. Polyneuritis.

Drowsiness and asterixis are common, as are stupor and coma. Convulsions also are not unusual. In fact, one may find any type of encephalopathy associated with hepatic failure. Reflexes may change frequently. For instance, one may get a positive Babinski in the morning, and in the afternoon the Babinski may disappear. The electroencephalogram may be helpful in differentiating hepatic decompensation from other types of encephalopathy, but the patterns are often non-specific. While at autopsy the brain sometimes shows an increase of astrocytes, there is really no known specific brain lesion that causes the encephalopathy. Peripheral neuritis with hyperesthesia, including pain, is often seen and is thought to be caused by the direct effect of ethanol and/or malnutrition.

Changes in pulmonary system

Hydrothorax is one of the more frequent chest findings in chronic liver disease. It occurs in 5% to 10% of people who have ascites. It is caused by the ascitic fluid filtering directly through the diaphragm into the right hemothorax. Occasionally one will see hemothorax, probably as a result of the bleeding tendencies such patients develop (Christian, 1927; Meigs, 1954). There are often arteriovenous fistulae in the lungs and pleurae.

Changes in gastrointestinal system

Abnormalities in the gastrointestinal tract are many, as noted in Table 3. Varices of the esophagus and stomach with portal hypertension may be considered almost a part of the disease. Splenomegaly is evident in about 50% of cases (Ratnoff and Patek, 1942). Peptic ulcer is more common than in normal individuals, and its incidence is increased even more after shunt surgery (Lipp and Lipsitz, 1952; Patek, 1963). Fetor hepaticus, which in the past was frequently encountered and which indicates liver decompensation, is now quite rare (Challenger and Walshe, 1955). This clinical finding may be less because of the frequent administration of such antibiotics as neomycin in the management of the disorder.

Ascites, a peritoneal transudate, is a hallmark of cirrhosis with which we are all familiar. We will not go into the mechanisms involved. Recently two types of peritonitis in cirrhotics have been reported, one caused by pneumococci (Epstein, Calia, and Gabuzda, 1968) and the other by enteric organisms (Conn, 1964), the diagnosis being made by culture of ascitic fluid. Pancreatitis is found clinically in about 5% of cirrhotics, according to Lipp and Lipsitz (1952).

Hepatoma is a rather common complication of cirrhosis, especially of the post-necrotic type. Recent studies by Alpert, Wogan and Davidson (unpublished data) in Uganda have demonstrated that certain fungal toxins, particularly aflatoxins from the aspergilli, may cause cirrhosis and hepatoma in animals. These aflatoxins are

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TABLE 2

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<th>Changes in Hematopoietic System</th>
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<td>Faulty Storage</td>
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<td>Fibrinogen</td>
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<td>Blood Loss</td>
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<td>Production of Abnormal Complexes (Macroglobulins)</td>
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<td>Poor Absorption</td>
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<td>Thrombocytopenia—Platelet Aggregation</td>
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<td>Clotting Defects</td>
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<td>Folic Acid Deficiency</td>
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<td>Acanthocytes</td>
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<td>L. E. Cells</td>
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<td>Fibrinolyisins</td>
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<td>Immunoglobulin A Deficiency</td>
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TABLE 3

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<th>Changes in Gastrointestinal System</th>
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<td>Fetor Hepaticus</td>
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<td>Pancreatitis</td>
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<td>Splenomegaly</td>
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<td>Abdominal Hernia</td>
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<td>Peritonitis: Spontaneous. Pneumococcic. Peptic Ulcer Hepatoma</td>
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found in the soil and in certain foods such as ground nuts. This brings up the important questions: Are there other toxins that may produce cirrhosis and hepatoma? Is fungal toxin, for instance, present in alcohol itself, and is this the factor that causes its toxic effect on the liver? Some recent work suggests this. One of the most interesting studies on hepatoma is by Lee in England (1966). In this study he reviewed a large group of alcoholic patients, all of whom had a finely granular type of cirrhosis. When these patients stopped drinking, within five or six months, according to his study, they developed very large nodules in the liver, a nodular type of cirrhosis; 16 (55%) of the 29 individuals who gave up drinking developed hepatoma, but only 9 (16%) of the 56 who continued to drink developed hepatoma. It was of interest that fewer females had hepatomas, but at the same time a much smaller percentage of women discontinued alcohol. We have recently seen advanced post-necrotic cirrhosis with very large nodules in which a transition from benign to malignant cells is clearly evident. Why neoplasia develops in this type of environment is still unclear (Parker, 1957; Kay, 1964; Miyai and Reubner, 1963).

Changes in blood chemistry

Abnormal blood chemical findings are frequently seen, and hyperlipemia is one of the more common, especially in biliary cirrhosis. Hyponatremia is almost the rule in the decompensated ascitic, in spite of the fact that the individual has increased body sodium; when the sodium level is 125 mm or below, it may be a poor prognostic sign (Hecker and Sherlock, 1956; Eisenmenger et al., 1950; Pecikyan, Kanzaki, and Berger, 1967; Galambos and Wilkinson, 1962). Hypomagnesemia may be found in the advanced cirrhotic and may be responsible for some of the encephalopathies that occur. A marked increase in globulins, especially in the gamma fractions, is frequently seen in chronic liver disease, and probably more often in post-necrotic cirrhosis. Hypoglycemia may occur in severe liver disease, as the liver is depleted of glycogen. It may also occur in hepatoma, but the cause of it is still unclear. Hypoalbuminemia with values below 2 mg/100 ml is not unusual in advanced chronic liver disease. This plays an important role in the fluid retention so often observed.

Changes in temperature regulation

Continued low-grade fever is not too rare, and, while cirrhosis predisposes to many types of infection, it is now known that the hepatic pathology itself may produce a febrile response (Tisdale and Klatskin, 1960). In animals a hepatic pyrogen has been isolated, and it is likely that in some instances in humans the damaged liver releases a pyrogen capable of inciting fever in the host. In animals this hepatic pyrogen is easily extractable and may be suppressed by glucocorticoids.

Of 80 patients studied by Klatskin and Tisdale, 58 had unexplained fever. It was concluded that this was probably a reflection of the diseased liver and not a result of any other complications.

Miscellaneous changes

One rather commonly observed finding is parotitis. There is no specificity to the pathological changes found in the parotid glands. The glands are usually bilaterally enlarged, firm and rubbery, and are probably more commonly seen in cirrhotics associated with alcoholism (Ratnoff and Patek, 1942; Patek, 1963).

Unexplained abdominal pain may be present in patients with uncomplicated cirrhosis. At times it may be quite severe. Several authorities have observed that it is more commonly found in patients who have concomitant ascites. It should be emphasized that advanced cirrhosis may be present with concomitant portal hypertension, although the patient may have no subjective symptoms. Rolleston and McNee (1929), in a study of 167 postmortem examinations of cirrhotics, noted that 87 of these had had minimal or no symptoms. McCartney (1933) found no evidence that the patient had symptoms in 35% of individuals who died with cirrhosis.

Summary

An effort has been made to present a panoramic view of cirrhosis. It has been indicated that many agents may initiate an intrahepatic process which may progress to advanced cirrhosis, that the characteristic abnormalities may cause both functional and pathologic multi-system changes, and that these encompass almost every body structure. As the altered structural and physiological changes progress, hepatic decompensation develops, and this often is terminal.

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The Relation of the Intestinal Cell Surface to Vitamin B₁₂ Absorption*

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Department of Medicine, Boston University School of Medicine, Massachusetts 02118

I appreciate the opportunity to join in this symposium and be here in Richmond. You are learning today that small bowel function can now be studied in many different ways, but during this presentation I would ask you to restrict your attention to events occurring right at the absorptive surface of the intestinal cell. There are valid reasons for taking such a narrow view. To be absorbed by the small bowel, any nutrient must traverse the membranous structure which lines the absorptive surface of the intestinal epithelial cells, and, thus, all absorptive mechanisms depend upon the characteristics of this surface membrane. You have seen how the intestinal mucosa is thrown into fingerlike projections called villi, and Dr. Sessions showed some elegant movies of the movement of these villi. You should recognize that the surface of the individual cells lining these villi is further increased by tiny projections known as microvilli. The microvilli consist of a trilaminar unit membrane and a central core of proteinaceous filaments which extend into the cytoplasm and unite to form a terminal web. Lying on the outer surface of the membrane is a mucopolysaccharide surface coat or fuzz. Ito (1965) has shown alkaline phosphatase activity in the fuzzy surface coat by histochemical techniques adapted for the electron microscope. Adenosine triphosphatase activity is also present in brush borders. Various disaccharidases which digest sucrose, maltose, and lactose to yield their constituent monosaccharides are located at the surface of the intestinal cell. Miller and Crane (1961) demonstrated them in brush border preparations. Eichholz (1967) subsequently showed these sugar-splitting enzymes to be more precisely located.

The brush border area of the cell consists of microvilli, terminal web, and a small portion of apical cytoplasm. When intestinal cells are ruptured according to the method of Miller and Crane (1961), this portion of the cell can be isolated by repeated centrifugation. Brush borders can then be further disrupted to yield a relatively pure preparation of microvillous membranes separated out by density gradient centrifugation (Eichholz and Crane, 1965). Since it is now possible to examine brush borders and microvillous membranes separately from the rest of the intestinal cell, it is safe to predict that you will be hearing more and more about the composition and function of these surface structures. In fact, considerable information is already available concerning this "absorptive-digestive" surface. Alkaline phosphatase activity has been localized to the brush border area by many workers and to the microvillous membrane by Eichholz (1967). Ito (1965) has shown alkaline phosphatase activity in the fuzzy surface coat by histochemical techniques adapted for the electron microscope. Adenosine triphosphatase activity is also present in brush borders. Various disaccharidases which digest sucrose, maltose, and lactose to yield their constituent monosaccharides are located at the surface of the intestinal cell. Miller and Crane (1961) demonstrated them in brush border preparations. Eichholz (1967) subsequently showed these sugar-splitting enzymes to be more precisely located.

*Presented at the Thirty-Ninth Annual McGuire Lecture Series, November 9-10, 1967, Medical College of Virginia, Richmond.
in microvillous membranes, and Johnson (1966) has more recently suggested their location at the fuzzy surface coat. Leucine aminopeptidase and cholesterol ester hydrolase are found in the brush border of absorptive cells. In terms of function, McDougal and others in Crane’s laboratory (1960) showed that isolated brush borders actively transport glucose against the concentration gradient. We have recently demonstrated an intrinsic factor-mediated attachment of Vitamin B₁₂ to intestinal brush borders and microvillous membranes (Donaldson, MacKenzie, and Trier, 1967; MacKenzie et al., 1967). The work will be described in some detail as an illustration of the kinds of information to be obtained from an examination of these surface structures.

Precisely how intrinsic factor (IF) promotes Vitamin B₁₂ absorption is not known, but it is now clear in all species studied that intrinsic factor, to be effective, must first bind the vitamin in a macromolecular complex. Furthermore, IF-dependent B₁₂ absorption occurs predominantly from distal rather than proximal portions of the intestine. In man, for example, it is generally accepted that the terminal ileum is the major site for B₁₂ absorption. These observations suggest that a specific binding site or receptor for the IF-vitamin B₁₂ complex may be present on the surface of ileal absorptive cells. To investigate this problem, we incubated radioactive Vitamin B₁₂ with brush borders and microvillous membranes isolated from either the proximal or distal half of hamster small bowel.

Each of these preparations was isolated from either the proximal or distal half of hamster intestine. Hamster gastric juice served as the source of intrinsic factor. When these preparations were obtained from the proximal half of hamster intestine, IF regularly depressed rather than enhanced B₁₂ uptake. On the other hand, IF regularly enhanced uptake when these preparations were taken from the distal half of the intestine. Specific uptake in terms of picograms B₁₂ per milligram of tissue nitrogen is much greater for microvillous membranes than for brush borders or mucosal homogenates. These observations directly demonstrate intrinsic factor acting at the absorptive surface of distal but not proximal intestine. The IF-mediated attachment to membrane occurs rapidly, is not increased with prolonged incubation, is not affected by temperature changes from 7°C to 37°C, and does not require glucose or oxygen. These findings are consistent with a physical adsorption of the IF-B₁₂ complex onto the membrane rather than with any energy-requiring enzymatic process. This physical attachment is very specific, however, since only hamster and rat intrinsic factor stimulate B₁₂ uptake by hamster brush borders. Intrinsic factor from hog, dog, rabbit, guinea pig, and man are all ineffective. Vitamin B₁₂ binders present in serum, saliva, or tissue extract which do not contain intrinsic factor activity fail to promote attachment. Uptake is maximal only when all of the B₁₂ in the incubating medium is bound in a macromolecular complex with hamster intrinsic factor. Thus, the binding site on the hamster intestinal membrane shows considerable specificity for this IF-B₁₂ complex.

It seemed to us that if a specific receptor to the IF-B₁₂ complex did indeed reside on the very surface of the intestinal cell, then it might be possible to make an antibody to such a receptor. Thus, we injected into rabbits pure preparations of microvillous membranes obtained from either the proximal or distal half of hamster intestine (MacKenzie et al., 1967). When brush borders were incubated with normal rabbit serum, IF-mediated attachment of B₁₂ proceeded normally. However, when these brush borders were first incubated with antisera to distal microvillous membranes, the effect of intrinsic factor was completely and regularly blocked. On the other hand, serum from rabbits injected with proximal microvillous membranes was not inhibitory. Thus, distal, but not proximal microvillous membranes, contained something which induced in rabbits a serum inhibitor to the attachment of the IF-B₁₂ complex to the surface of hamster intestine.

Antisera to distal microvillous membranes capable of inhibiting IF-mediated uptake contain no antibodies against intrinsic factor itself or against the IF-B₁₂ complex as determined by three different methods. Furthermore, the inhibitory factor could be absorbed from antisera by distal brush borders but not proximal brush borders or by intrinsic factor. These findings are consistent with the idea that distal microvillous membranes induced an antibody directed against the intestinal receptor for the IF-B₁₂ complex.

To determine the nature of the observed inhibition, we incubated increasing quantities of IF-B₁₂ with brush borders in the presence of either normal rabbit serum or antisem to distal microvillous membranes. The results showed that the inhibitory factor could be overcome by an excess of IF-B₁₂ and suggested that inhibition is competitive. Presumably the inhibitory factor competes with IF-B₁₂ complex for a common binding site or receptor on the microvillous membrane.

Several lines of evidence suggest that this inhibitory factor is an antibody. First, it was produced in serum by injecting a foreign substance into rabbits. In addition, chromatography identified the inhibitory factor as an immunoglobulin. When rabbit antisera was eluted from a Sephadex G-200 column, the usual three protein peaks were obtained. When these peak fractions were tested for inhibitory activity, only the second 7S globulin peak produced significant inhibition. Chromatography on diethylaminoethyl-cellulose and immunoelectro-
phoresis confirmed that immunoglobulin G contained the inhibitory factor. I have indicated thus far that distal, but not proximal microvillous membranes induce an antibody against the intestinal binding site for the IF-B$_{12}$ complex and that this inhibiting antibody can be absorbed by distal, but not proximal brush borders.

We wondered whether proximal and distal microvillous membranes could induce the formation of other antibodies and, if so, whether cross-reactions between proximal and distal would occur. Double diffusion in agarose gel shows distinct precipitin lines when antibodies to proximal and distal microvillous membranes are tested against brush border extracts from the distal and proximal halves of the intestine. Under these conditions, cross-reactions between proximal and distal tissues occur. Thus, both proximal and distal microvillous membranes contain similar antigens, but only distal microvillous membranes contain an antigen capable of producing the inhibitory factor. In order to locate the site of antibody attachment to the intestinal cell, we stained hamster intestine either with fluorescein-labelled normal rabbit serum or with fluorescent antiserum to microvillous membranes. Fluorescent antiserum was clearly localized only in the brush border area of the intestinal cell. More precise localization of the site of the antigen-antibody reaction was attempted. Immunoglobulin G from control rabbits and from rabbits injected with microvillous membranes was conjugated with ferritin, and the ferritin-labelled antibodies were incubated with hamster intestine. The location of ferritin particles was then identified with the electron microscope by Dr. Jerry Trier.

Numerous dense ferritin particles were localized to the fuzzy mucopolysaccharide surface coat of the microvilli but not to the unit membrane itself. This localization is not surprising when one consid-

ers the fact that, in general, mucopolysaccharides are potent antigens. This observation raises the possibility but does not provide evidence that the inhibitory factor in rabbit antisera may also localize to the fuzzy surface coat. At present we are investigating this possibility, since, using immunologic techniques, we should now be able to distinguish whether the intestinal receptor for IF-B$_{12}$ is situated on the mucopolysaccharide surface coat.

I have tried to indicate that it is now possible to separate out events occurring at the surface of intestinal absorptive cells. Previous work has shown that intestinal brush borders contain diverse enzymatic activity and that the surface of the intestinal cell is a site for active glucose accumulation. Furthermore, brush border and microvillous membrane preparations now provide direct evidence that intrinsic factor acts at the intestinal cell surface. These studies directly support the concept that a specific receptor for the IF-B$_{12}$ complex is located on the surface of ileal, but not jejunal absorptive cells. Studies with antibodies against pure preparations of microvillous membranes verify the existence of this receptor and provide an approach to its further localization and characterization.

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Amino-Acid Absorption*

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Most, if not all, ingested protein is transported across the gut as its constituent amino acids. Thus, the way the gut handles amino acids is an important physiologic problem, and their mishandling by the gut in disease states represents an important threat to general body activities. I propose to discuss some of the knowledge that has been gathered over the past 10 to 15 years concerning the mechanisms of amino acid absorption. The data to be presented have been gathered from three major kinds of studies: the studies of in vitro model systems, physiologic studies of humans, and studies of patients with syndromes involving specific amino absorption abnormalities. Some of these data have been accumulated in our own laboratory; much of what I have to present is the work of other investigators.

In Vitro Studies on Gut

Figure 1 shows an experimental technique which has become a classic model for the study of transport of various substances, not only amino acids. This is the everted gut sac. The technique involves making everted gut segments from the intestines of small animals. The animal’s gut is removed as quickly as possible, cleaned, turned inside out so that the mucosa is on the outside, and small sacs made of the segments of the gut. The sacs are then placed in various bathing solutions containing the substances to be tested.

Figure 2 shows the kinds of results one can obtain with this kind of method. As indicated by the dotted line, the concentration of the substance tested, in this case the amino acid, L-tryptophan, was the same on both the mucosal and the serosal sides. The measurement of the concentration changes at various time intervals following the placing of the substance in the sac is an indication of what movement is going on. Under aerobic conditions (the box on the left), the mucosal concentration of tryptophan (black column) decreased after an hour’s incubation, and the serosal concentration increased. Remember that the initial concentration was the same on both sides. Anaerobically, when oxygen was not allowed in the flask, concentration against a gradient no longer occurred.

The importance of aerobic, energy-dependent systems for transport of L-tryptophan from the mucosal surface to the serosal side is shown in Figure 3. In this study, tryptophan initially was placed only on the mucosal side, in contrast to the previous study. None was on the serosal side. After an hour’s incubation aerobically, not only had some tryptophan been transported from the mucosal to the serosal side, but there also now existed a concentration gradient between these two sides. By contrast, anaerobically the transfer from the mucosal to serosal side was very much diminished, and no concentration gradient had been developed.

Figure 4 shows an experiment with a substance for which there is no active transport mechanism. The test substance was pyridoxine. In the upper bar is shown the situation when pyridoxine was placed at equal concentration on both sides of the everted gut sac. There was no concentration gradient developed from the mucosa to serosa either aerobically or anaerobically. The bottom set shows the situation when the pyridoxine was placed only on the mucosal side. Transfer was slow; no concentration gradient developed, and it did not make any difference whether the environment was anaerobic or aerobic. This further confirms the idea that energy-dependent systems are of significance in transporting tryptophan, whereas they are not of significance in transporting pyridoxine.

By the use of this in vitro technique, unphysiologic as it may sound, much information has been gathered as to the transport of amino acids. Table 1 shows some data obtained with a series of amino acids using the everted gut sac technique. A concentration gradient > 1.00, as indicated in the third column, indicates that there was accumulation of these amino acids on the serosal side as compared to the mucosal side, the amino acids having been in each instance placed in equal concentrations on both sides of the membrane at the start of the experiment. It is obvious that different amino acids behave in somewhat different

* Presented at the Thirty-Ninth Annual McGuire Lecture Series, November 9-10, 1967, Medical College of Virginia, Richmond.
manners. In addition to testing single amino acids, mixtures of amino acids and amino acids in which parts of the amino acid molecules have been modified have also been studied.

On the basis of such studies in the everted gut sac, it appears that certain types of amino acids are transported by specific mechanisms. It is presently thought that there exist separate specific transport mechanisms for the monocarboxylic neutral amino acids; for dibasic amino acids, such as lysine, arginine, and ornithine along with the neutral amino acid, cystine; for the dicarboxylic amino acids; and, possibly, for proline, hydroxyproline and, questionably, glycine.

Figure 5 shows a sketch of how amino acids might interact with a membrane carrier. This scheme applies, most likely, to the neutral amino acids. The carboxyl, the hydrogen, and the alpha amino groups all are quite specific, since substitutions at any one of these sites decrease the transport rate of the amino acid. The question mark next to the pyridoxal phosphate indicates that certain studies suggest that this substance may be an important co-factor in neutral amino acid transport. The longer the side group, indicated here as the R, the more lipophilic the substance is and the greater the affinity of the amino acid for the lipid carrier or the lipid membrane. It has been shown in the group of neutral amino acids that those with a longer side chain, i.e., those with a more lipophilic side chain, compete preferentially for transport with those with shorter side chains.

Thus, on the basis of these animal in vitro experiments, of which this has been only a brief resume, there has evolved a concept of amino acid absorption as being one in which there are several specific carrier mechanisms involved.

Studies on Human Gut

In order to study amino acid absorption in humans, different tech-

Fig. 1—Everted sac method of Wilson and Wiseman. Tied sac of everted hamster jejunum containing 1 ml of fluid. X 2.64 (Reprinted with permission from T. H. Wilson, Intestinal Absorption. Philadelphia: W. B. Saunders, 1962, p. 33.)

Fig. 2—Transport of ³H-labelled L-tryptophan by everted loops of rat small intestine. The interrupted line indicates initial concentration on both sides of the sac. The final concentrations in each of two experiments are shown by the columns. Note that at the end of one hour there is a concentration gradient between the inside of the sac (serosal side) and outside (mucosal side) when incubation is aerobic. This does not occur anaerobically. (Reprinted with permission from The Scientific Basis of Medicine Annual Reviews. London: The Athlone Press, 1963, p. 172.)
TABLE 1

Rates of Transference of Amino Acids and Concentration Gradients Developed by Sacs of Everted Intestine

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Rate of transference (µl./mg dry wt./hr)</th>
<th>Concentration gradient developed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Praline*</td>
<td>14.0 ± 3.2</td>
<td>2.08 ± 0.18</td>
</tr>
<tr>
<td>Threonine</td>
<td>12.0 ± 2.7</td>
<td>1.90 ± 0.28</td>
</tr>
<tr>
<td>Alanine</td>
<td>11.5 ± 3.5</td>
<td>1.82 ± 0.32</td>
</tr>
<tr>
<td>Glycine*</td>
<td>10.1 ± 2.4</td>
<td>1.65 ± 0.19</td>
</tr>
<tr>
<td>Serine</td>
<td>8.8 ± 2.2</td>
<td>1.61 ± 0.19</td>
</tr>
<tr>
<td>Valine</td>
<td>8.2 ± 2.7</td>
<td>1.42 ± 0.19</td>
</tr>
<tr>
<td>Histidine*</td>
<td>5.3 ± 2.8</td>
<td>1.42 ± 0.22</td>
</tr>
<tr>
<td>Hydroxyproline</td>
<td>6.3 ± 1.8</td>
<td>1.36 ± 0.14</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>5.4 ± 2.2</td>
<td>1.24 ± 0.11</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>4.0 ± 1.2</td>
<td>1.19 ± 0.09</td>
</tr>
</tbody>
</table>

* These results are taken from Wiseman (1955) and are included in this table for the sake of completeness. The experiments were performed under identical conditions.

Adapted with permission from *J. Physiol.* 133: 628, 1956.

Fig. 3—Typical everted loop experiments using ³H-labelled L-tryptophan initially on the outside of the sac (mucosal side) only. Aerobically active transport results in the development of a concentration gradient. Anaerobically only diffusion occurs. (Reprinted with permission from *The Scientific Basis of Medicine Annual Reviews.* London: The Athlone Press, 1963, p. 173.)

Techniques must be developed. Aside from the difficulty of obtaining pieces of human gut to make everted gut sacs, such sacs could not be used in the same way as sacs from hamster gut. Besides possible species differences in transport, from the practical standpoint there is the fact that, in the larger species, such as dog, the muscle wall is so thick that penetration of substance from mucosa through to the serosal side is ineffective. In humans two major kinds of approaches have been used. One method is to take pieces of gut at operations, or by biopsy, and perform in vitro studies. I shall discuss these studies later, mainly in regard to certain disease states. Another method is to perfuse the gut under more or less physiologic conditions. Perfusion experiments to study intestinal absorption of amino acids and other substances in the human have been widely used. In general the method is as follows: A subject is intubated with a tube which most often contains two holes, a perfusing hole and an aspirating hole, some distance down the gut. The test substance is then perfused along with a non-absorbable reference marker, polyethylene glycol being one that is commonly and popularly used today. The disappearance from the gut lumen of the substance to be studied in relation to changes in marker concentration as a measure of water absorption has been used as a test for absorption of the specific substance being studied. Thus, if the concentration of amino acids drops during the test, in relation to the concentration polyethylene glycol, some of the amino acid has been absorbed during the period of perfusion.

Table 2 shows the kind of data obtained when the absorption of glycine was studied by the perfusion method. As the glycine concentration in the perfusion fluid was increased, the amount absorbed, ex-
pressed as millimoles per 15-centimeter gut segment perfused, also increased. The increase in the amount absorbed, however, was not linearly related to the increase in the glycine concentration of the perfusion fluid. The rate of glycine absorption reached a limiting value. This is illustrated in Figure 6, which shows the results of studies of L-isoleucine absorption in two subjects. Plotted are millimoles absorbed against the concentration of isoleucine perfused. As the concentration increased, the amount absorbed also increased, but the absorption rate tailed off as the concentration increased. If simple diffusion were the process involved, one would expect a straight-line relationship between the concentration perfused and the amount absorbed. Instead, this curve suggests saturation kinetics and is, thus, consistent with the idea that some sort of active transport process is taking place.

Figure 7 shows the results of transport fluids in which the gut was perfused with solution containing both glycine and L-alanine. At the bottom of Figure 7 are indicated the concentrations of each substance for any one study. For example, “fifty” means that a 50 mM solution of glycine and a 50 mM solution of L-alanine were perfused together. Three patients were

<table>
<thead>
<tr>
<th>Perfused (mM)</th>
<th>Absorbed (mmoles/15 min/15-cm gut segment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1 ± 0</td>
</tr>
<tr>
<td>25</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>50</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>75</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>100</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>150</td>
<td>10 ± 3</td>
</tr>
<tr>
<td>300</td>
<td>15 ± 4</td>
</tr>
</tbody>
</table>

Adapted with permission from J. Clin. Inv. 45: 1435, 1966.

Fig. 4—Everted loop experiments using ³H-labelled pyridoxine HCl. The interrupted line indicates the initial concentrations. With equal concentrations on both sides of the sac initially (upper figure), there is no active transport for no concentration gradient develops. When pyridoxine is only present on the outside (lower figure), it diffuses across into the serosal fluid equally well whether incubation is aerobic or anaerobic. (Reprinted with permission from The Scientific Basis of Medicine Annual Reviews. London: The Athlone Press, 1963, p. 174.)

Fig. 5—Hypothetical interaction of neutral amino acid with the membrane carrier. (Reprinted with permission from T. H. Wilson, Intestinal Absorption. Philadelphia: W. B. Saunders, 1962, p. 123.)
studied, and on each several experiments were performed. It can be seen that, at every concentration studied, the absorption of alanine, shown by clear bars, was always greater than the absorption of glycine. If passive diffusion process alone were involved, the two substances would not be expected to so drastically influence each other's absorption.

Another way of testing whether or not substances share a common site for absorption involves holding the concentration of one substance constant but changing the concentration of the other substance. The first is then called the inhibitor; the second, the test substance. Figure 8 shows a Lineweaver-Burk plot, a double reciprocal plot in which $1/S$ is the reciprocal of the concentration perfused, and $1/V$ represents the reciprocal of the amount absorbed. The solid line connecting these open circles indicates the absorption of glycine at various concentrations when perfused alone. The dotted line indicates the absorption of glycine in the same subject when perfused at various concentrations together with 150 mM L-alanine as the inhibitor. This line is displaced upwards and to the left; in other words, the absorption of glycine is decreased in the presence of the inhibitor, L-alanine. However, as the concentration of glycine is increased, the line tends to meet the line representing absorption of glycine alone; it intercepts the vertical axis at the same place. The fact that the lines under these two conditions arrive at the same point is consistent with a form of inhibition called competitive inhibition, that is, where two substances compete for the same transport mechanism or for the same binding site. If the inhibition were not competitive, that is, if the binding site were being poisoned in some way by the inhibitor, then the intercepts would not be expected to be at the same place. These studies in humans with glycine and alanine...
are, thus, consistent with the studies in everted gut sacs in in vitro preparations. They indicate that these particular amino acids are absorbed by active transport mechanisms.

The influence of the lipophilic side chain, suggested by the everted gut sac experiments, can also be seen when three amino acids are tested together. Figure 9 shows the results of perfusing together at equimolar concentrations: isoleucine, alanine, and glycine. Isoleucine was always absorbed faster than alanine, which, in turn, was always absorbed faster than the glycine.

On the basis of these studies and the everted gut sac studies, one would then predict that L-isoleucine would be a very effective inhibitor of glycine absorption. Surprisingly, however, this was not found. Figure 10 shows Lineweaver-Burk plots of glycine absorption. The solid line indicates glycine absorption when glycine alone was perfused. The small-dash line indicates the effect of 150 mM alanine; the large-dash line is the effect of isoleucine as an inhibitor at 150 mM. No inhibitory effect by the L-isoleucine on glycine absorption was seen. Isoleucine, as indicated previously, certainly is transported more quickly than is glycine. These studies show that isoleucine is not, however, as effective an inhibitor of glycine absorption as is alanine. This might well indicate that there are two steps in amino acid transport. One would be a postulated entry step in which the length of the lipophilic chain is important and in which the longer side chain of isoleucine produces greater affinity with the lipid membrane, enabling it to be transported more quickly. The second step, perhaps an exit step, may be the specific carrier mechanism and, indeed, may be the rate-limiting step for the process. Isoleucine and glycine do not appear to share this later step.

Fig. 8—Results of perfusing glycine alone (straight line). Results of perfusing glycine in the presence of 150 mM L-alanine (dash line). (Reprinted with permission from J. Clin. Inv. 45:1437, 1966.)

Fig. 9—Results of perfusing isoleucine (dotted line), alanine (dash line), and glycine (straight line) together at equimolar concentrations.
In Vitro Studies on Gut Mucosa

In vitro studies on the distribution ratios of amino acids between gut mucosa and bathing solution may shed additional light on specific transport mechanisms for certain amino acids. Results obtained from such studies are shown in Table 3. By distribution ratio is meant the concentration of amino acid taken up by the mucosa in relation to the concentration in the bathing fluid. A distribution ratio \(>1.00\) indicates that tissue accumulated amino acids. For instance, when lysine alone was incubated with the tissue, the distribution ratio was \(4.57 \pm 1.84\). When lysine was incubated together with either arginine or cystine, the distribution ratios found were considerably and statistically decreased as compared to the situation when lysine alone was incubated. Glycine incubated together with lysine had no effect on lysine uptake by the tissue, indicating that lysine, arginine, and cystine very likely form one family, glycine not being part of that family. Cystine incubated alone gives a distribution ratio of \(3.61 \pm 0.85\). When incubated together with lysine as the inhibitor, the distribution ratio is less. Glycine again has no effect on cystine distribution in the tissue.

Thus, many interesting results have already been obtained from in vitro studies and studies on normal humans in regard to amino acid absorption of the gut. Much remains to be learned about the normal processes by which amino acids are handled by the gut. The fact that various mixtures of amino acids may be handled differently can be of considerable metabolic significance, since it has been shown that lack of certain amino acids in the diet results in poor growth rates and poor wound healing rates. It appears that the body has to be presented with certain optimum mixtures of amino acids for optimum function. With this, then, as some indication of what

![Fig. 10—Results of perfusing glycine alone (straight line). Results of perfusing glycine in the presence of 150 mM alanine (dotted line). Results of perfusing glycine in the presence of 150 mM isoleucine (dash line).](image)

### TABLE 3

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Inhibitor</th>
<th>No. of Tests</th>
<th>Distribution Ratio</th>
<th>Significance of difference from control (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-lysine</td>
<td>None</td>
<td>12</td>
<td>4.57 ± 1.84</td>
<td></td>
</tr>
<tr>
<td>L-lysine</td>
<td>L-arginine</td>
<td>4</td>
<td>1.13 ± 0.22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>L-lysine</td>
<td>L-cystine</td>
<td>4</td>
<td>1.77 ± 0.26</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>L-lysine</td>
<td>Glycine</td>
<td>3</td>
<td>4.81 ± 0.40</td>
<td>&gt;0.6</td>
</tr>
<tr>
<td>L-cystine</td>
<td>None</td>
<td>10</td>
<td>3.61 ± 0.85</td>
<td></td>
</tr>
<tr>
<td>L-cystine</td>
<td>L-lysine</td>
<td>4</td>
<td>2.31 ± 0.35</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>L-cystine</td>
<td>Glycine</td>
<td>3</td>
<td>3.63 ± 0.96</td>
<td>&gt;0.9</td>
</tr>
</tbody>
</table>

Adapted with permission from Science 143: 483, 1964. Copyright by the American Association for the Advancement of Science.
TABLE 4
Uptake of L-Lysine-C\textsuperscript{14} and L-Cystine-S\textsuperscript{35} by Gut Mucosa from Normal Subjects and Patients with Cystinuria

<table>
<thead>
<tr>
<th></th>
<th>Distribution Ratio (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L-lysine</td>
</tr>
<tr>
<td>Normal subjects</td>
<td>13.4 ± 1.91</td>
</tr>
<tr>
<td>Cystinuric subjects</td>
<td>1.4 ± 0.26</td>
</tr>
</tbody>
</table>

(p < 0.001) (p < 0.001)

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TABLE 5
Evidence Favoring a Jejunal Transport Defect for Tryptophan in Hartnup Disease

1. Ingested L-tryptophan can be recovered in feces of patients but not of normal subjects.
2. Plasma levels of tryptophan after ingestion of the amino acid rise more slowly and remain high for a longer time in cases of Hartnup disease than in normal subjects.
3. Bacterial breakdown products of tryptophan are excreted in greater amounts and for longer periods after ingestion of the amino acid in Hartnup disease patients than in normal subjects.

Adapted with permission from Quart. J. Med. 29: 415, 1960.

TABLE 6
Evidence Concerning a Defect of Tryptophan Absorption in the Blue Diaper Syndrome

1. Tryptophan content of stools, before and after loading with tryptophan, higher in patients than controls.
2. Excess urinary excretion of indolic metabolites, especially after tryptophan feeding, formed in lower gut by action of bacteria on unabsorbed tryptophan.
3. I. V. tryptophan in patient increased metabolites of kynurenine pathway several times as compared to similar amounts of oral tryptophan. No such discrepancy between oral and I. V. doses found in controls.
4. Plasma tryptophan levels following oral loading low in patients compared with controls.


The state of the art is at the moment insofar as amino acid under normal conditions is concerned, let us turn briefly to some abnormalities of amino acid absorption.

Abnormalities of Amino Acid Absorption

Cystinuria

The first entity to be discussed is cystinuria. This is a genetically determined disorder in which the outstanding clinical manifestation is the formation of cystine renal calculi. It has been known that aminoaciduria occurs in cystinuric patients. Only over the last few years, however, has it been determined that a specific amino acid transport defect can also be seen in the gut. The results of in vitro studies with isotopically labeled lysine and cystine incubated with gut mucosa obtained from cystinuric and normal subjects are shown in Table 4. The amino acid distribution ratios in the gut of normal subjects were considerably and statistically significantly higher than the distribution ratios seen when the gut mucosa of cystinuric subjects was used. Thus, cystinuric gut mucosa has a specific defect for concentrating these particular amino acids. The defect in cystinuria is confined to the basic amino acids, since other amino acids that have been studied do not show the transport difficulties.

Another way of studying the amino acid absorption defect in cystinuria is to follow the fate of ingested ornithine and lysine. When this was done, amino acid was found in the stool, and various breakdown products of the amino acids were found in both the feces and the urine. In normal subjects, feeding of these amino acids was not accompanied by similar changes. These findings indicate that, in cystinuria, the amino acids are inefficiently absorbed by the small bowel and, thus, can be acted on by colonic bacteria. Thus, both
by in vitro experiments with gut mucosal biopsies and by feeding of amino acids, it can be demonstrated that there is a defect in handling of basic amino acids by patients with cystinuria.

Hartnup Disease

Another disorder in which a defect in amino acid absorption can be demonstrated is a rare genetic disorder named Hartnup disease. This disorder is characterized by a pellagra-like skin rash, a severe but reversible cerebellar ataxia, various psychiatric manifestations, and an aminoaciduria involving alanine, serine, threonine, and a number of other amino acids. Glycine, aspartic acid, glutamic acid, lysine, arginine, and ornithine are excreted in normal amounts in Hartnup disease. Some observations concerning gut absorption abnormality in Hartnup disease are summarized in Table 5. Ingested tryptophan can be recovered in the feces of patients but not of normal subjects. After ingestion of the amino acid, plasma levels of tryptophan rise more slowly and remain high for a longer time in cases of Hartnup disease than in normal subjects. Furthermore, after ingestion of the amino acid, bacterial breakdown products of tryptophan are excreted in greater amounts and for a longer period in Hartnup disease patients than in normal subjects. These findings could be explained by an enzymatic block along the metabolic sequence from tryptophan to nicotinic acid or by an abnormal intestinal environment supporting a peculiar gut flora which deranged the metabolism of the host. The most likely explanation, however, is that the kidney and the gut share a metabolic abnormality for the transport of amino acids. Certainly further studies need to be done before this hypothesis can be accepted.

Except for the deficiency of nicotinic acid and the production of the pellagra-like rash in Hartnup disease, the effect of amino acid absorption deficiency in both Hartnup disease and cystinuria is not presently clear on a clinical basis. It has been suggested that growth may be retarded in these patients, and calculations of the amount of amino acid lost in the urine and potentially unabsorbed indicate that the amino acid intake may be marginal. This may be particularly true for lysine, which is a limiting amino acid in many nutritional studies.

Blue Diaper Syndrome

Another disorder in which there also appears to be a defect in tryptophan absorption is the so-called Blue Diaper Syndrome, a familial disease in which hypercalcemia and nephrocalcinosis are associated with a defect in the intestinal transport of tryptophan (Table 6). Bacterial degradation of the tryptophan leads to excessive indole production and, thus, to indicanuria, which, on oxidation to indigo blue, causes peculiar bluish discoloration of the diaper; hence, the picturesque name.

Phenylketonuria

In phenylketonuria there also may be a defect in absorption of amino acids, at least as tested by oral administration of arginine. Figure 11 shows the appearance of arginine in blood after feeding labelled arginine to a patient with phenylketonuria. The appearance of the label in the serum was very much delayed, and the radioactivity levels achieved were very much lower than in a normal control subject. This is, at best, only preliminary suggestive evidence for malabsorption, since appearance curves by themselves are subject to many
AMINO-ACID ABSORPTION

variables and cannot be taken entirely as indicating an amino acid transport defect. That this defect may, however, be a reversible phenomenon is demonstrated in Figure 12, where line A is the appearance of radioactivity in the blood after feeding of radioactive leucine in a patient with phenylketonuria. Line B shows the results of the same test in the same subject after a period of phenylalanine deprivation. Considerable improvement in amino acid uptake is seen, matching the observation on the normal control subject of the same age. These data and other that have been gathered in terms of the effect of phenylalanine on amino acid transport into brain tissue suggest that the abnormality leads to a metabolic block of amino acid transport which may be reversible in nature.

Effect of Galactose on Amino Acid Transport

As a final example of a disorder in which amino acid transport by the gut may be affected, the effect of galactose has been studied. Table 7 shows that the gut mucosa of rats, prefed 30% galactose for several months, had a diminished uptake of various amino acids compared to control values. Prefeeding rats with a variety of other sugars such as glucose, xylose, and ribose has no effect, whereas prefeeding with fructose has a minimal effect. The possible implications for the human disease, galactacemia, are evident.

Conclusion

In concluding, it is of some use to compare the criteria for active and passive transport presented in Table 8. Although somewhat more sophisticated subdivisions of this table can be made, I think it is useful to review the data in this way. In active transport, the substance is transported from a fluid of a lower concentration to that of a higher; passive diffusion movement is only from a higher concentration to a lower. The rate of transport is not necessarily related to molecular volume, whereas in passive diffusion, by and large, smaller molecules diffuse at a greater rate. In active transport there is stereospecificity, that is, stereoisomers are generally transported less efficiently than the naturally occurring compounds. It suggests that there is a specific kind of receptor site to which the transported molecule must be attached. In active transport the rate of transport is not proportional to the concentration gradient. It shows the saturation phenomenon at high substrate concentrations. This does not occur in passive diffusion. In active transport one can show competitive inhibition by related compounds; the rate of transfer is unaffected by the presence of closely related compounds in passive diffusion. Transport rate is reduced by metabolic poisons when material is actively transported, whereas the rate of transfer is not greatly affected in passive diffusion.

TABLE 7

<table>
<thead>
<tr>
<th>Intestinal Slices</th>
<th>Distribution Ratios</th>
<th>Hydroxy-L-proline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L-alanine</td>
<td>L-valine</td>
</tr>
<tr>
<td>Control</td>
<td>4.0 ± 0.6</td>
<td>4.2 ± 0.9</td>
</tr>
<tr>
<td>Galactose-fed</td>
<td>2.8 ± 0.8</td>
<td>2.3 ± 0.3</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Adapted with permission from Nature 205: 700, 1965.
TABLE 8
Comparison of Active Transport and Passive Diffusion

<table>
<thead>
<tr>
<th></th>
<th>Active Transport</th>
<th>Passive Diffusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relation to concentration gradient</td>
<td>Transport from fluids of a lower to a higher concentration occurs</td>
<td>Movement only from fluids of a higher to a lower concentration</td>
</tr>
<tr>
<td>Relationship to apparent molecular volume in compounds of similar type</td>
<td>Speed of transport not necessarily related to apparent molecular volume</td>
<td>Smaller molecules in general diffuse at a greater rate</td>
</tr>
<tr>
<td>Stereospecificity</td>
<td>Stereospecific. Stereoisomers in general are transported less readily than naturally occurring compounds</td>
<td>Rate of transfer of stereoisomers identical</td>
</tr>
<tr>
<td>Saturation of system at high concentrations</td>
<td>Rate of transfer not proportional to concentration gradient and approaches a limiting maximal value</td>
<td>Rate of transfer proportional to concentration gradient and no limiting maximal rate occurs</td>
</tr>
<tr>
<td>Competitive inhibition by related compounds</td>
<td>Rate of transfer reduced by simultaneous transport of a related compound sharing the same transport system</td>
<td>Rate of transfer unaffected by the presence of closely related compounds</td>
</tr>
<tr>
<td>Non-competitive inhibition</td>
<td>Transport reduced by metabolic poisons—e.g. dinitrophenol</td>
<td>Rate of transfer not affected unless obvious cellular damage occurs</td>
</tr>
</tbody>
</table>


unless cellular damage has occurred.

Thus, there are many characteristics by which one can attempt to assess whether or not materials are transported passively or require a specific energy-dependent system for their movement. There is reason to believe on the basis of the evidence presented here, on the basis of in vitro studies with everted gut sacs and other tissue preparations, on the basis of physiologic perfusion studies, and, perhaps most significantly, on the basis of studies in various disease states with specific amino acid transport defects, that amino acid transport by the gut is an active process which may be affected by a variety of inhibitors in pathophysiologic states. We are just beginning to understand the importance of these defects in human metabolism.

References


Pathogenesis of Hepatic Encephalopathy

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Severe parenchymatous diseases of the liver, both acute and chronic, are frequently associated with hepatic encephalopathy. This term is preferable to that of hepatic coma, as it encompasses the whole spectrum of changes from bizarre alterations of behavior to various degrees of disturbance of consciousness, as well as protein neurologic manifestations. In many instances it is a reversible phenomenon, occurring either spontaneously or as the result of various therapeutic agents. The puzzling feature of hepatic encephalopathy is the discrepancy between dramatic clinical features and paucity of histopathological changes in the brain. The only histological changes encountered with regularity in patients with this entity are diffusely swollen and enlarged astrocytes (Adams and Foley, 1953). With no macroscopic or microscopic changes to account for cerebral dysfunction, it is likely that hepatic encephalopathy is caused by profound, yet undefined metabolic abnormalities.

A relationship between ammonia toxicity and cerebral dysfunction has been suspected since the discovery of “meat intoxication” in Eck fistula dogs. The clinical relationship between liver disease, cerebral dysfunction and disordered ammonia metabolism was established by Gabuzda, Phillips, and Davidson (1952) and confirmed by others.

Sources of Ammonia

The major sources of ammonia in humans are the bowel and the kidney. Ammonia is produced in the bowel by the action of bacterial and digestive enzymes on protein and urea, the major contributor probably being the right colon (Gryska and Barsamian, 1958; Silen et al., 1955; Webster, Davidson, and Gabuzda, 1958). In patients with cirrhosis, the upper small bowel, normally sterile, is contaminated by bacteria, thus contributing significantly to ammonia production (Martini et al., 1957). The reason for abnormal bacterial growth in the upper small bowel in patients with cirrhosis is not known.

Hydrolysis of the urea is an additional source of ammonia. About 25% of the endogenous urea is not excreted by the kidney but is hydrolyzed in the gastrointestinal tract at a constant rate of 0.3 mg per hour to a total of 7.2 gm of urea daily. This is equivalent in nitrogen content to 11 gm of NH₄Cl or 18 gm of protein (Walser and Bodenlos, 1959). As patients with advanced cirrhosis in terminal stages frequently develop azotemia secondary to renal failure, it is easy to visualize how hydrolysis of this excess urea in the gut could precipitate hepatic coma. All these factors, when coupled with a larger protein load, either secondary to bleeding or high protein diet, can contribute significantly to the occurrence of hepatic encephalopathy.

The second major source of ammonia is the kidney. Ammonia is produced in the kidney by deami-
nation of glutamine and other amino acids, resulting in higher renal vein ammonia. The difference between renal vein and arterial ammonia is even more pronounced in patients with cirrhosis, particularly if they are potassium deficient, on diuretic therapy, or both (Weil-Malherbe, 1950; Owen et al., 1960). The mechanism for preferential secretion of ammonia into the renal vein is not known. With acetazolamide therapy, increase in renal vein ammonia was coupled with equivalent fall in urinary ammonia and a rise in urine pH. The latter may explain the shift in partition of ammonia between urine and renal vein.

The role of hypokalemia in an increased renal production of ammonia is not understood, but it can be prevented by correction of potassium deficit. In patients with liver disease who are frequently rendered hypokalemic with injudicious use of diuretics, this mechanism may be responsible for the precipitation of hepatic encephalopathy.

Removal Mechanisms and Distribution of Ammonia

Ammonia is detoxified in the liver by forming urea through the Krebs-Henseleit cycle. As a result of the marked collateral circulation and hepatocellular disease, the removal of ammonia by the liver in patients with cirrhosis is diminished. Very rarely in cases of massive fulminating liver failure, the urea cycle does not function at all, resulting in decreased blood urea nitrogen levels.

The removal of ammonia from the blood and tissues can also be accomplished through the reaction in which \( \alpha \)-ketoglutarate combines with ammonia to form glutamic acid (Weil-Malherbe, 1950). Through the process of transamination, \( \alpha \)-ketoglutarate can again become available either to combine with more ammonia or to continue in the tricarboxylic acid cycle (Fig. 1). Furthermore, glutamic acid can react with ammonia to form glutamine in the reaction requiring ATP.

These reactions, thus, probably serve as a defense against accumulation of ammonia, and at the same time offer a reasonable explanation for frequent elevation of glutamic acid and glutamine in patients with chronic liver disease. Exhalation of ammonia via the lungs is a minor mechanism of ammonia removal.

As a consequence of hepatic parenchymal disease and porto-systemic shunts, the nitrogenous compounds from the portal blood bypass the liver and reach systemic circulation, resulting in the elevation of blood ammonia. Blood ammonia, however, is not a good index of “ammonia toxicity” for several reasons. First, blood ammonia correlates poorly with the state of consciousness (Moore, Strohmeyer, and Chalmers, 1963). Secondly, in the congenital defects of urea biosynthesis, blood ammonia levels may be much higher than those found in patients with hepatic coma with no neuropsychiatric manifestations (Russell et al., 1962). Finally, the experiments by Warren et al. (1960; Warren, 1958; Stabenau, Warren and Rall, 1959) have shown that another variable, pH, might play an important role in the pathogenesis of hepatic coma. They observed that the differential toxicity of various ammonium salts depended upon the change in the pH of the blood induced by a given salt.

Ammonia, in the blood or any biological fluid, exists in two forms: ammonium ion \( (\text{NH}_4^+) \) and free ammonia \( (\text{NH}_3) \). According to the Henderson-Hasselbalch equation—

\[
\text{pH} = pK_a + \log \frac{\text{NH}_3}{\text{NH}_4^+}
\]

the relative proportion of each component is directly dependent upon pH. Knowing the \( pK_a \) of ammonia (about 9.15), total blood ammonia (obtained by any standard microdiffusion test) and pH, one can calculate the relative proportion of ionized \( (\text{NH}_4^+) \) and un-ionized free ammonia \( (\text{NH}_3) \). The relative proportions of un-ionized ammonia at pH's of 6, 7, 8, and 9.15 are approximately 0.1%, 1%, 10%, and 50%. According to present concepts, only un-ionized free ammonia is capable of crossing the cell membrane. At the normal blood pH only a small proportion of ammonia is in this form. The change of pH to the alkaline side will increase the proportion of un-ionized ammonia \( (\text{NH}_3) \) and, thus, theoretically, increase the chance of inducing hepatic encephalopathy.

According to the pH gradient distribution hypothesis, distribution of ammonia from blood to tissue seems to be dependent upon a pH difference between the two compartments. If a membrane, such as

![Fig. 1—Ammonia detoxication reaction involving \( \alpha \)-ketoglutaric Acid. The transamination reactions involving glutamic acid are also presented.](image-url)
the blood-cerebrospinal fluid barrier, is permeable only to un-ionized ammonia, the concentration gradient which normally exists between extra- and intracellular fluid should be maintained, depending upon the pH in each compartment. With the change of this gradient, the redistribution of un-ionized ammonia occurs, providing the change takes place on one side of the membrane only or both sides in the opposite direction. Consequently, an increase in extracellular pH, such as in metabolic alkalosis, will both increase the relative proportion of un-ionized ammonia and facilitate its transfer across the membrane, resulting in a rise of intracellular ammonia.

Respiratory and metabolic alkalosis are frequently encountered in patients with cirrhosis. Respiratory alkalosis with hyperventilation is the most frequent acid-base disturbance in these patients. The underlying mechanism for its occurrence is not clear. This type of alkalosis, however, does not cause significant change in the pH gradient, as carbon dioxide diffuses freely across the cell membranes and, consequently, no change in ammonia distribution occurs. Conversely, in patients with metabolic alkalosis, the pH gradient will be increased, as the pH change will occur on one side of the membrane only, thus resulting in redistribution of ammonia. Hypokalemic, hypochloremic alkalosis accompanied by intracellular acidosis frequently occurs in patients with cirrhosis who are treated with potent diuretics. As a result of metabolic alkalosis, formation of un-ionized ammonia (NH$_3^°$) is favored, and its transport across the membrane is facilitated. This mechanism may be partially responsible for the frequent precipitation of hepatic encephalopathy in cirrhosis on this therapy.

Whether aforementioned theoretical considerations play an important role in the pathogenesis of hepatic coma is not yet entirely clear, largely because no direct method for measuring un-ionized ammonia (NH$_3^°$) is available.

Clinical trial with the intention of implementing these theoretical considerations in patients with hepatic coma was not successful (Warren et al., 1960). The administration of 0.1N HCL intravenously for a brief period of time, in an attempt to lower the pH, did not lead to any improvement in the state of consciousness. The duration of this clinical trial was very short and, therefore, inadequate to fully test this hypothesis.

Pathogenetic Mechanisms

Hepatic encephalopathy can be classified either as spontaneous (endogenous) or induced (exogenous). As far as the pathogenesis is concerned, this classification is somewhat arbitrary. It only implies that exogenous coma is precipitated by a variety of factors (Table 1) and, consequently, does not necessarily imply extremely poor liver function. Conversely, spontaneous or endogenous coma usually implies active, sometimes fulminating liver disease, occurring without presence of precipitating factors and, consequently, carries a worse prognosis.

The pathogenetic mechanisms involved in hepatic encephalopathy are not known. It is generally accepted, but not proven, that either ammonia or a nitrogenous substance liberating ammonia is in some way associated with the syndrome of hepatic encephalopathy. In addition to, or because of disordered ammonia metabolism, patients with hepatic encephalopathy have a markedly altered metabolism of glucose and oxygen. This is manifested by the accumulation of various intermediates of tricarboxylic acid (TCA) cycle, pyruvic and lactic acid in the blood and in the spinal fluid. Furthermore, brain oxygen uptake and cerebral blood flow in these patients are decreased between 25% and 50%. All of these facts indicate that profound metabolic biochemical changes take place in these patients, some of them affecting cerebral energy metabolism. Such disturbance of cerebral energy metabolism is now thought to be the basis of neurological changes in ammonia intoxication. Neurologic findings of hepatic encephalopathy such as asterixis, decerebrate rigidity, hyperpnea and coma suggest malfunction of the specific structures in the base of the brain and possibly their cortical connections (Magoun, 1963; Plum and Posner, 1966).

The understanding of biochemical disorders involved in hepatic coma was hampered by the impossibility of studying coma in humans. Most of the available data

<table>
<thead>
<tr>
<th>TABLE 1</th>
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<tr>
<td>Gastrointestinal Bleeding</td>
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<tr>
<td>Excessive Amounts of Nitrogenous Substances (Dietary Protein, Ammonium Salts, Urea, Amino Acids)</td>
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<td>Azotemia</td>
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<td>Diuretics</td>
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<td>Metabolic Alkalosis With or Without Hypokalemia</td>
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<tr>
<td>Acute Infections</td>
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<tr>
<td>Sedatives, Hypnotics, Analgesics (Chloral Hydrate, Paraldehyde, Barbiturates, Opiates)</td>
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<td>Major Surgical Procedures</td>
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<td>Massive Paracentesis</td>
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are from in vitro experiments or animal models. Thus, it must be stressed with great emphasis that extrapolation of these experiments to human situations should be done with reservation and due caution.

Bessman and Bessman (1955) proposed a hypothesis which attempted to link the effect of excess ammonia in the brain with the effectiveness of the TCA cycle. The common link is α-ketoglutarate, an important intermediate of the TCA cycle. They suggested that, when an excess of ammonia is presented to the brain, depletion of α-ketoglutarate takes place through its reaction with ammonia to form glutamic acid. This reaction (Fig. 2) is catalyzed by glutamic dehydrogenase and uses DPNH as an electron donor. Diversion of α-ketoglutarate from the TCA cycle diminishes the rate of formation of the succeeding members of the cycle. It is apparent that this will interfere with oxygen utilization and reconstitution (or formation) of adenosine triphosphate (ATP), an overall process known as oxidative phosphorylation. The formation of ATP will be diminished in direct proportion to the depletion of α-ketoglutarate.

The second line of defense in the process of ammonia detoxication in the brain is offered by the reaction in which glutamic acid reacts with ammonia to form glutamine (Fig. 2). This reaction requires ATP for energy and is catalyzed by glutamine synthetase. As a result of the accentuated formation of glutamine, this compound is elevated in the cerebrospinal fluid and blood in patients with hepatic coma.

Worcel and Erecinska (1962) have also shown that the addition of ammonia to rat liver mitochondria inhibited oxygen uptake when α-ketoglutarate and pyruvic acid were used as substrates. They postulated that the inhibitory action of ammonia on the respiration of rat liver mitochondria is due to competition for DPNH between glutamic dehydrogenase and electron chain transport (Fig. 2).

If these theoretical considerations are correct, when an increased amount of ammonia crosses the blood-brain barrier and reacts with α-ketoglutarate and glutamic acid, brain ATP levels should be decreased as a result of the combination of inadequate formation and excessive utilization. For the same reasons, α-ketoglutarate would be expected to be low. Schenker et al. (1967; Shorey, McCandless, and Schenker, 1967) measured ATP and α-ketoglutarate in the brain of rats who were given toxic doses of ammonium acetate. ATP and α-ketoglutarate were measured in the rapidly frozen base and cortex of the brain. Their results indicate that the ATP levels were unchanged in the cortex but definitely decreased at the base of the

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**Fig. 2—Metabolic interrelationship between ammonia metabolism and TCA cycle in the brain. The dotted (. . . . .) line illustrates metabolic derangements of the TCA cycle postulated to occur as a result of excess ammonia. (Adapted with permission from Gastroenterology 49:699, 1965.)**
brain, with ammonia concentration equal in both parts. These experiments offer for the first time in vivo evidence of the toxic effect of ammonia on energy metabolism in the brain. At the same time, a measurable decrease of phosphocreatine at the base was observed. Glucose and glycogen decreased significantly in both base and cortex.

The mechanism for this selective depletion of ATP at the base of the brain is not known, but it could be due to the impaired formation of ATP, its increased utilization, or the combination of both. The significance of selective ATP and phosphocreatine depletion at the base of the brain is not known. However, the available evidence suggests that one-third depletion of ATP is deleterious in anoxic rats (Dahl and Balfour, 1964). The role of ATP in the maintenance of proper cerebral function is also not known, though it has been postulated that energy provided by ATP may be required for proper transmission of nerve impulses (Skou, 1965). The relationship between the state of consciousness and ATP depletion was not clarified in these experiments.

The determination of α-ketoglutarate did not demonstrate any depletion of this intermediate either in the cortex or at the base of the brain. It is possible, however, that the current methods cannot assess small cerebral pools in highly differentiated compartments. These results, as well as the failure of ammonia to interfere in vitro with either respiration or phosphorylation, are not compatible with Bessman's hypothesis.

McKhann and Tower (1961) showed that the addition of ammonium chloride to the brain cortical slices caused significant reduction of oxygen uptake when pyruvate or α-ketoglutarate were used as a substrate. They concluded that ammonium interferes with oxidative decarboxylation of pyruvate and α-ketoglutarate. This observation offers another possible hypothesis applicable to the human situation. Other factors such as deficient acetyl coenzyme A, metabolic inhibitors—either not detoxified in the liver or produced as a result of ammonia intoxication—and medium chain fatty acids are a few possible candidates which have not yet been adequately studied.

Though a great step forward, these experiments have not yet offered a new concept relating to the pathogenesis of hepatic encephalopathy.

The concerted efforts to explain hepatic encephalopathy from the standpoint of disordered ammonia metabolism heretofore have not been rewarding. Geiger (1958), however, in his experiment with perfused cat brain, has opened new avenues and new approaches. He found that cat brain preparations remained viable and were able to perform normal neurological and biochemical functions for a prolonged period of time with the liver inserted into the circulation. This was not the case when the brain preparation was perfused with the red cell suspension in the Ringer solution devoid of organic substances normally present in blood. This emphasizes the importance of the metabolic interrelationship between the liver and the brain. The implication of these experiments is that normal brain function depends upon a normally functioning liver, which may supply the brain with some important metabolic precursors such as cytidin and uridin which the brain is itself incapable of producing (Webster, 1965). In patients with cirrhosis, this metabolic interrelationship might be disturbed, resulting in abnormal brain function.

A repeat of the Geiger experiments with these thoughts in mind, as well as new and systematic approaches in other areas, might offer additional insight into the pathology of this fascinating and perplexing entity, which is so inadequately understood at the present time.

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The Surgical Management of Hirschsprung's Disease

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Etiology, Early History

Aganglionic megacolon, or Hirschsprung's disease, is characterized by constipation dating from birth. The symptoms of constipation or obstruction are due to a lack of parasympathetic innervation resulting from congenital absence of nerve ganglia of the myenteric and submucosal plexuses of Auerbach and Meissner. The aganglionic segment begins at the anus and extends proximally for a varying distance. In most cases of Hirschsprung's disease, the aganglionic segment involves only the rectum and the lower sigmoid colon. Occasionally, the aganglionic segment may involve most or all of the colon and, rarely, the lower small intestine.

The first description of Hirschsprung's disease is usually credited to a Dutch surgeon, Frederici Ryuschii, who published a detailed report in Latin in the seventeenth century (Benson et al., 1962; Jayle, 1909). Hirschsprung's Classical Paper was delivered before the Berlin Congress for Children's Diseases in 1886. He considered the dilated colon to be the primary cause, although he did mention that the rectum was not dilated and seemed slightly narrower than the normal rectum and that the mucosa of the colon was ulcerated, inflamed and edematous (Hirschsprung, 1887). The true etiology remained generally poorly understood in spite of correct observations of several authors dating from 1901, indicating the primary defect. Swenson and Bill (1948) have described a sphincter-saving curative proctectomy removing the aganglionic segment of the colon. This operation was a significant milestone in the treatment of this previously incurable disease. However, it is associated with a high instance of postoperative complications. Several authorities have expressed concern regarding long-term complications of proctectomy for this as well as other diseases (Clausen and Davies, 1963) and have expressed a preference for preservation of the rectum in histologically benign condition (Duhamel, 1960; Martin and Altemeier, 1962). A low anterior abdominal resection has been preferred by some (State, 1952). Others have proposed a rectosigmoid myotomy similar to the Ramstedt procedure and Heller operations (Martin and Burden, 1927). Duhamel (1960) has described a procedure which consisted of excluding the rectum, leaving it in place, and establishing an oblique, end-to-side anastomosis at the skin level. This procedure avoided the complications of proctectomy and, at the same time, the anastomosis was low enough to avoid residual symptoms of Hirschsprung's disease. There were some complications relating to accumulation of stool in the blind rectal stump. Also, the sensory urge to defecate, derived from the tense, filled rectum, was lost, since the rectum was completely bypassed by the procedure. This operation was further modified by lengthening the anastomosis as a long, side-to-side attachment of the colon to the rectum (Martin and Altemeier, 1962). Soave (1964) has recently described...
an operation which preserves the muscular wall of the rectum but removes the mucosa and does a pull-through procedure, bringing the ganglion-containing colon through the muscular sleeve of the rectum, which is kept in place.

Operative Procedure

The operation which we now employ is a modification of the above procedures (Martin and Caudill, 1967). The rectum is left in place but is completely included in the fecal stream, so that the sensory function is preserved. Dissection within the pelvis is minimal, thus reducing the risk of injury to the small nerve fibers going to the bladder and to the ejaculatory mechanism. The anastomosis can be performed at a low level without disturbing the internal sphincter.

The operation (Fig. 1) is carried out with the patient in the lithotomy position. The abdomen is opened through a generous, left paramedian rectus-retracting approach. The rectum is divided just above the peritoneal floor, with the anastomosis clamps being placed obliquely, preserving a longer segment of rectum anteriorly. Resection of the aganglionic sigmoid and descending colon is then carried out proximally to a level where adequate numbers of ganglion cells are identified microscopically on frozen section by the pathologist. Following resection, the end of the colon is then closed and inverted with interrupted silk sutures. The ends of the sutures are left long so that they may be grasped with the clamp to be subsequently inserted through the rectum.

The presacral space is next opened and dissection carried downward in the midline posterior to the rectum, using the finger to facilitate dissection until the level of the pelvic diaphragm is reached.

The surgeon then proceeds to the perineal portion of the operation. The anus is gently dilated and the rectum thoroughly irrigated with sterile saline solution followed by a mild antiseptic. Employing the Bovie electrosurgical unit, a transverse incision is made around the posterior half of the rectal wall at the apex of the anal crypts. The wall of the rectum posteriorly is dissected gently from the levator muscles, and the presacral space previously dissected from above is then entered. A long, curved hemostat is inserted from below into the presacral space, and the lower end of the colon is grasped and drawn through the wound in the posterior rectum. Several fine catgut sutures are placed about the circumference of the opening of the posterior rectal wall, and an open, end-to-side anastomosis is created from the end of the colon to the posterior wall of the rectum. After the sutures closing the end of the colon have been removed, a long spur-crushing clamp of the Mikulicz variety is inserted, with one prong in the rectum and one in the colon, and a clamp inserted the full length of the rectal stump.

The surgeon then returns to the abdominal part of the operation, and an open anastomosis is carried out between the end of the rectal stump and the side of the adjacent colon.

Fig. 1—A. Following resection of the aganglionic portion of the colon, the proximal end is placed in the presacral space and the end of the colon anastomosed to the posterior wall of the rectum 0.5 cm proximal to the mucocutaneous junction through an incision in the posterior wall of the rectum made transversely through the dilated anus.
B. Anastomosis from the proximal end of the rectum to the side of the adjacent colon is created, employing the open anastomosis technique.
C. The remaining colo-rectal septum is obliterated by means of a spur-crushing clamp which is inserted from below by an assistant while the surgeon is completing the proximal anastomosis. This permits placement of the clamp under direct vision before the anterior row of the anastomosis has been completed. The clamp is tightened in position and obliterates the remaining colo-rectal septum within a period of three to five days.
D. The appearance of a completed anastomosis.
E. The interior of the completed anastomosis.
colon. After the posterior half of the anastomosis has been completed, the spur-crushing clamp, which was previously inserted through the perineal approach, is inserted further by the assistant and placed under direct vision by the surgeon, so that the clamp includes the entire length of the septum to assure its complete ablation. The anterior half of the anastomosis is completed in the usual manner. If possible, the peritoneal floor is repaired above the anastomosis. A proximal diverting colostomy has generally been established previously and is considered advisable in most cases.

Results

This operation has been employed in 17 children with Hirschsprung's disease. Their ages were from one to four years. All have been followed up at regular intervals, with the longest follow-up being four years. One child developed mild pelvic cellulitis, fever and leukocytosis following discharge from the hospital. The cellulitis cleared promptly with oral antibiotics. Three children developed mild abdominal distention and diarrhea, interpreted as enterocolitis, one to three months following closure of the colostomy. Their symptoms cleared following anal dilatation. Mild constipation in two children has required occasional medication. None have had problems related to accumulation of stool in the rectum. The degree of bowel control has been most gratifying. The children have from one to three bowel movements daily, and their underclothing remains clean during the interim. Clinically, the results of this operation have been satisfactory.

Summary

The operative technique which we have employed for 17 children with Hirschsprung's disease is presented. The procedure offers certain advantages over the original Duhamel operation and over the classic Swenson operation. It can be recommended as a safe operation for Hirschsprung's disease, and, in our experience, has given satisfactory results.

References


Abstracts of Theses for Graduate Degrees

Medical College of Virginia, June, 1967

Respiratory Gas Tensions and Flow of Pulmonary Lymph in Anesthetized Dogs

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Thirty-seven samplings of right duct lymph, thoracic duct lymph, arterial blood, mixed venous blood and expired air, made separately and simultaneously in 59 out of 300 anesthetized mongrel dogs, showed a mean value of 61 mm Hg for the right duct lymph PO$_2$ and 37 mm Hg in the thoracic duct lymph. The pulmonary lymph PO$_2$ was probably higher than that in the right duct and close to alveolar air, since the right duct carries lymph from non-gas exchanging areas as well as from alveoli. Lung lymph could not be collected in the remaining 241 dogs because of technical difficulties and communication between the right and thoracic ducts (in 16% of the animals). The possibility of diffusion of oxygen through the walls of the right duct and smaller lymphatics made interpretation of the PO$_2$ values for lymph of the right duct difficult. A counter-current diffusion exchange mechanism of oxygen between pulmonary arterial blood and pulmonary lymphatic vessels appeared possible. The right duct lymph PCO$_2$ was similar to arterial PCO$_2$. The right duct lymph pH was higher than the pH of either the thoracic duct lymph or the arterial blood.

Pulmonary lymph flow was estimated to constitute about 40% of lymph flow from the right duct. Simultaneous measurements in 28 dogs showed mean values of 4.5 and 24.6 ml/hr, respectively, for control lymph flow from the right and thoracic ducts. The flow from the right duct was related to tidal volume but not to the body weight of the animal. It was found that left atrial pressures below those generally believed to cause pulmonary edema, e.g., 10 or 15 mm Hg, increased flow from the right duct. This finding could only be explained by the presence of interstitial tissue pressure around the pulmonary capillaries. Hypoxia induced by low O$_2$ breathing and i.v. administration of dinitrophenol increased the pulmonary lymph flow, probably because of increased capillary permeability. Alloxan increased lung lymph flow markedly. Hyperoxia (100% O$_2$ breathing) did not change the rate of pulmonary lymph flow.

A Linkage Map of Seven Loci in the X-Chromosome of Drosophila tropicalis

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Department of Biology and Genetics

The order of seven mutant genes, one dominant and six recessive, in the X-chromosome of Drosophila tropicalis has been determined and a linkage map constructed. The recombination frequencies for some of the seven genes varied in different crosses, which may have been a result of variability associated with the different strains used in this study.

When compared to the X-chromosome linkage map of some other species that also possess a metacentric X-chromosome, the region of 36 units mapped in D. tropicalis appears much shorter and thus probably represents only a segment of the whole X-chromosome. This is almost certainly true because of the small number of sex-linked mutants known in this species. As more sex-linked mutants are found, the map distance will likely increase because of the probability of finding genes near both ends of the chromosome.

Comparison of the X-chromosomes of D. tropicalis and D. willistoni was made, using the presumed homologous mutants. Three schemes have been presented to illustrate inversion sequences that may have taken place between these two species. Both overlapping and included inversions would result in a similar gene order.

A Study of Some Factors Affecting Starch Swelling and their Relationships to Tablet Disintegration

JAMES THOMAS INGRAM, M.S.
Department of Pharmacy

The project was planned to study some factors influencing starch swelling at 37° C. and their relationships to tablet disintegration. The swelling capability of starch was determined by microscopic examination. One hundred or 200-grain diameters were measured for each environmental condition. The mean grain diameters were compared in various full
factorial experimental designs to determine whether changes in environmental conditions produced significant changes in grain diameters. This statistical significance was determined by calculation of analyses of variance and application of F-ratio tests.

To determine the relationships between starch swelling and tablet disintegration, tablets of various materials were prepared with cornstarch as the disintegrant. These tablets were studied for the correlations between compressional force, disintegration time, starch grain damage, void space and elastic recovery.

Cornstarch and amioaca starch, when submersed 5 to 30 minutes in simulated gastric fluid USP (SGF), had greater increases in grain sizes than when submersed in distilled water.

The effects of the individual components of SGF were examined. Changes in pH had little effect on swelling. Salts affected swelling, with polyvalent cationic salts (MgCl₂ and AlCl₃) producing greater diameter increases than monovalent cationic salts (NaCl and Na₂SO₄). Ionic concentration did not produce an effect on swelling. There was no statistically significant swelling demonstrated by pepsin or surfactants in the submersion medium.

No significant difference in swelling was demonstrated between the various time intervals. However, when unsubmersed starches were included in the analyses, significant differences were shown between the unsubmersed starches and the starches slurried for five minutes and longer.

The swelling of starch grains was in the order of 5% to 10% increase in mean grain diameter. This was calculated to represent a volume increase of about 1.1% to 5.5% in a tablet containing 10% cornstarch. Since most tablets contain more than 5.5% void space, this did not seem to be a large enough change to cause the tablet to rupture.

The literature review indicated that damaged starch grains will swell in cold water. This was demonstrated by experiments with cornstarch damaged by ball milling. Submersion of the starch samples that had been ball milled from 10 to 48 hours produced increases in grain diameters of 40% to 80%.

Since damaged starch grains were shown to have an increased swelling capability in SGF at 37° C., the effect of the tableting procedure on starch grain damage was investigated. Damage to the grains, resulting from compression of pure cornstarch, was shown to be insignificant.

The effect of compressional force and hardness of the tablet ingredients was examined. In each formulation the starch grain damage increased as the compressional force was increased. The ingredient apparently had no effect on the direct proportionality of this relationship but did have an effect on the degree to which it occurred. There was no correlation between degree of starch grain damage and hardness of the ingredient. The crystalline form of the ingredient may exert a greater influence on starch damage than hardness. There was no correlation between starch damage and stress produced by elastic recovery of the tablet after removal of pressure. Contrary to what might be expected, there was an inverse relation between the amount of stress and the degree of starch grain damage for all of the formulations except aspirin.

There was no evident relationship demonstrated between disintegration time and starch grain damage. Other than compressional force, the inherent effect of the tablet ingredient was the only factor that appeared to affect disintegration.

The long accepted swelling mechanism of starch as a tablet disintegrant was not demonstrated in this study. The results of the investigation revealed no measurable correlations between starch grain damage and disintegration or between starch swelling and disintegration.

Circulatory Effect of Hypercapnia and its Role in the Production of the Vasodilator Response to Ischemia

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Department of Physiology

Despite extensive studies, the magnitude of the vasodilator effect of CO₂ on blood vessels in skin and muscle and its role in the local regulation of blood flow are still controversial.

In the present work the vasodilator effect of CO₂ in the human forearm was evaluated quantitatively, its mechanism of action was investigated, and its role in the production of the vasodilator response to ischemia was determined.

One hundred and twenty-seven experiments were carried out in 66 normal human volunteers. Forearm blood flow was measured by venous occlusion plethysmography. The vasoconstrictor effect of increased activity of sympathetic nerves and of circulating catecholamines was inhibited by the induction of alpha- adrenergic blockade through intra-arterial administration of phenoxybenzamine. In most experiments the dilator effect of circulating catecholamines was also eliminated by producing beta-adrenergic blockade through intra-arterial administration of propanalol or pronethalol.

Hypercapnia, produced by breathing 2.8% to 9% CO₂ in air, caused increase in forearm blood flow and decrease in forearm vascular resistance. In the steady state the decrease in vascular resistance during hypercapnia was related to the change in venous blood
PCO₂ or pH by a semilogarithmic relationship. In several experiments the decrease in vascular resistance during CO₂ breathing was produced by a short-lasting, small increase in vascular resistance.

In another series of experiments, the vasodilator effect of local hypercapnia was evaluated in the intact forearm in the following manner: isotonic saline equilibrated with 100% CO₂ was infused intra-arterially and its effect on forearm blood flow determined. During the infusion of saline with high CO₂ tension, forearm blood flow displayed a biphasic response consisting of a small initial decrease in flow followed by a more pronounced increase in blood flow. These experiments indicated that CO₂ has a local vasodilator effect on vessels of the human forearm.

Another series of experiments was undertaken to determine whether the stimulus for the CO₂-produced vasodilatation was increased PCO₂ or decreased pH. For this purpose, subjects breathed 7% CO₂ for two 10 minute periods. During one of these periods, decrease in pH in response to CO₂ breathing was abolished by the intravenous administration of sodium bicarbonate. The changes in forearm blood flow in forearm vascular resistance during the two periods were not significantly different, indicating that the stimulus for the CO₂-produced vasodilatation is increased PCO₂ rather than decreased pH.

The roles of local hypercapnia and local hypoxia in the production of the vasodilator response to ischemia were evaluated in the human forearm in the following manner: ischemia was produced by digital compression of the brachial artery, and the collateral flow was allowed to perfuse the forearm, while venous blood was withdrawn during the period of ischemia to determine the magnitude of the increase in PCO₂ and decrease in PO₂. The vasodilator response to ischemia was then compared to that produced by: a) increase in venous blood PCO₂ produced by CO₂ breathing, when the circulation was free; or b) decrease in venous blood PO₂, similar to that produced by ischemia, induced by breathing gas mixtures containing low concentration of oxygen, when the circulation was free; or c) increase in venous blood PCO₂ and decrease in venous blood PO₂, comparable to those changes produced by ischemia, induced by breathing gas mixtures having high concentration of CO₂ and low concentration of oxygen when the circulation was free. It was found that local hypercapnia accounted for about 50% to 60% of the vasodilator response to ischemia, while the contribution of local hypoxia was much less important, amounting to about 20% of the response to ischemia. It was found, furthermore, that the response to ischemia was not changed during 100% oxygen breathing, in spite of the fact that a substantial increase in venous blood PO₂ occurred, confirming the view that the contribution of local hypoxia to the response to ischemia is not pronounced.

The Initial Destruction of Intracellular Salmonella typhimurium

ANNA SYBIL RADCLIFFE, M.S.
Department of Microbiology

An experimental procedure was designed for the in vitro cultivation of macrophages infected with Salmonella typhimurium. Peritoneal macrophages from guinea pigs were permitted to phagocytize S. typhimurium and were cultured in suspension. At intervals, samples were taken for determination of total cell population and for quantitative recovery of cell-associated bacteria. The ratio of bacteria to cells was thus computed at each interval, and a curve was constructed representing the fate of intracellular parasites over a period of time after phagocytosis.

Two strains of S. typhimurium with different degrees of virulence against mice were compared by the above procedure. Data show that there is an initial destruction of intracellular bacteria of both strains. However, there is a difference in the extent of this intracellular destruction. With the avirulent strain there is a two-log decrease in the intracellular population, the minimum being reached in four hours after phagocytosis; whereas with the virulent strain there is only a 1.2 log decline in the intracellular population, its minimum being reached in three hours. After this period of decline, the surviving organisms in both strains begin to multiply.

Cell Culture of Oral Mucous Membrane Lesions

RAYMOND PETRIE WHITE, JR., Ph.D.
Department of Anatomy

The difficulty in recognizing the malignant potential of oral lesions is well appreciated by the clinician who faces this problem routinely. In an effort to provide information which may aid in determining the potential of these lesions, a tissue culture project was undertaken.

The specific aims of this project were: a) the development of a reliable in vitro system for the growth of normal cheek-pouch mucosa of the hamster, and b) the comparison of the growth of this normal mucosa with the growth of 9, 10-dimethyl-1, 2-benzanthracene (DMBA)-induced lesions of the hamster pouch.

The hamster mucosal lesions were produced by repeatedly painting the animal's cheek pouch with 0.5% DMBA, the opposite pouch in the same animal being used as a control. Biopsies of these areas were taken...
from the time the painting with the carcinogen began until obvious tumors appeared. A part of this tissue was preserved for histologic sectioning. The remaining tissue was explanted into Sykes-Moore chambers. These chambers were then incubated at 36±0.5° C. in a standard water jacket incubator. Eagle's Minimum Essential Medium with 10% Fetal Bovine Serum was used as a growth medium. Developing cell sheets in the Sykes-Moore chambers were observed with phase microscopy up to a 30-day maximum.

The tissue preserved for histologic sectioning was fixed in formalin, and routine H & E sections were prepared. Based on these sections, the tissue from the painted cheek pouches was divided into three categories: hyperplastic, early carcinomic, and late carcinomic. On this basis the tissues in culture could be compared.

Results of the pilot study indicated that primary cell cultures of hamster cheek-pouch epithelial cells could be repeatedly grown using a plasma clot explant culture technique. Twenty-four of 25 control cultures produced epithelial cell sheets in the first three post-explant days. Fibroblasts appeared later in these cultures, normally about the fifth post-explant day. These cells did not compromise the growth of the epithelial cells.

Cell size in this pilot study seemed to be a good indicator of growth activity. As long as small or medium cells could be found in the cell sheet, growth activity would continue until the next observation of that culture.

Epithelial cell sheets developed from the control tissue in a typical pattern. This process was followed from the initial appearance of cells around the explant until the continuity of the cell sheets was broken and the cells themselves had degenerated.

Three other experiments were carried out to confirm the results of the pilot study and to better compare the cultures of pathologic tissue with the cultures of normal tissue. Two of these experiments used a blind experimental design, so that the cultures from pathologic tissue were not identified until culture growth had terminated. The results of all three experiments were similar.

The cultures of normal tissue behaved much like cultures of normal tissue in the pilot study. Early epithelial cell sheets developed with a similar pattern whether they were from normal or pathologic mucosa. Cell size continued to be a good indicator of growth activity. The control cultures uniformly exhibited epithelial cell sheets in the first three post-explant days. However, none of the normal tissue samples produced cultures whose cell sheets persisted through the 30-day observation period.

In contrast many cultures of pathologic tissue showed poor growth or no growth at all. A large amount of granular debris was often seen accumu-
Book Review


Dr. Carvalho's book, VAGINAL CYTOLOGY, offers an inexpensive, brief, and clear survey of the various uses to which vaginal cytology is now applied. The book presents an orderly approach to the diagnostic uses to which cytology may be put in clinical endocrinology, complications of pregnancy, diagnosis of malignancy, and prediction of radiation sensitivity. The author's long experience, not only with cytology but also with clinical gynecology, is apparent from the clinical references sprinkled throughout the text and the clear descriptive terms pertaining to the smears themselves.

The text is easy to read, the descriptions are clearly stated, and the pathologist's usual addiction to descriptive terms such as "beer bottle caps" and "tennis rackets" is used to good advantage to describe some of the unusual cellular morphology. The illustrative material is obviously derived from a large collection which makes available both common findings and the rare and unexpected. Although the illustrations are well selected and abundant, the lack of color is a disadvantage, and they are somewhat disappointing in that they are poorly reproduced in many of the plates. The illustrations are so plentiful that one feels that perhaps the text could have been placed in juxtaposition with the illustrations, thereby proving of greater advantage to the reader.

All controversy regarding the uses of cytology other than for the diagnosis of malignancy is omitted. The skeptical physician who does not readily accept the cytologist's ability to diagnose such entities as ectopic pregnancy, impending labor, impending abortion, and radiation sensitivity will find little to convince him of the cytologist's claims. The material relating to these disorders is largely descriptive, and documentation of accuracy is not attempted. If not convincing, Dr. Carvalho's claims for the use of cytology in these entities is at least provocative.

The author and his associates are to be congratulated on producing a readable, convenient text that should find ready acceptance as a brief and clear review of the uses of cytology for those who are not actively engaged in this type of work. For the experienced cytologist and pathologist, Dr. Carvalho's observations regarding diagnosis of ectopic pregnancy, diabetes, and "phagocytic response" in patients undergoing radiation therapy should be a stimulus for further investigation.

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Professor and Chairman
Department of Obstetrics and Gynecology
Medical College of Virginia

THE SOCIETY
of the
SIGMA XI

Medical College of
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May 16, 1968

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Charles M. Caravati (*Cirrhosis: What Is It?*), assistant dean in charge of continuing education at the Medical College of Virginia, received his undergraduate education at the University of Richmond and his M.D. from MCV. After two years of internship and assistant residency at Providence Hospital in Washington, he returned to Richmond for private practice in internal medicine and gastroenterology and joined the faculty of MCV. Dr. Caravati has also served as chairman of the division of gastroenterology at MCV.

Robert M. Donaldson, Jr. (*The Relation of Intestinal Cell Surface to Vitamin B₁₂ Absorption*) is director of the gastrointestinal division at University Hospital in Boston. He received his B.S. from Yale University and his M.D. from the Boston University School of Medicine. Following his internship at Montreal General Hospital, he was assigned to general medical duty in the U.S. Naval Reserve. He returned to civilian life as resident at the Boston Veteran’s Administration Hospital and was subsequently a U.S.P.H.S. fellow at Peter Bent Brigham Hospital. Before assuming his present position, Dr. Donaldson was director of the patient study unit at University Hospitals in Madison, Wisconsin.

Bertram Fleshler (*Amino-Acid Absorption*), associate professor of medicine at Case Western Reserve University, received his A.B. degree from the University of Wisconsin and his M.D. from Boston University School of Medicine. After completing his internship at Massachusetts Memorial Hospital and assistant residency at Georgetown University Hospital, Dr. Fleshler served as a Captain in the United States Air Force Medical Corps. Before coming to Case-Western Reserve, he was a U.S.P.H.S. postdoctoral fellow at Massachusetts Memorial Hospital. Dr. Fleshler is also director of the division of gastroenterology at the Cleveland Metropolitan General Hospital.

Franz J. Ingelfinger (*Clinical Manifestations of Pancreatic Disease*), editor of THE NEW ENGLAND JOURNAL OF MEDICINE and former president of the American Gastroenterological Association, received his A.B. degree from Yale University and his M.D. from Harvard Medical School. Following house-staff training at Boston City Hospital, he spent a year at the University of Pennsylvania collaborating in the early studies of human small intestinal physiology. From 1940 to 1961 he was director of the division of gastroenterology at the Evans Memorial and Boston University School of Medicine. Before accepting his current position, Dr. Ingelfinger was director of the Boston University Medical Services at Boston City Hospital.
Lester W. Martin (The Surgical Management of Hirschsprung's Disease) is associate professor of surgery at the University of Cincinnati and director of pediatric surgery at the Cincinnati Children's Hospital. He received his undergraduate education at the University of Missouri, his M.D. at Harvard University, and his hospital training at The New York Hospital. Dr. Martin has held a variety of teaching positions at Cornell and Harvard Medical Schools, Brooke Army Medical Center, and Simmons College. At the present time he is also attending surgeon at the Cincinnati General Hospital.

Z. Reno Vlahcevic (Pathogenesis and Treatment of Hepatic Coma) is assistant professor of medicine at the Medical College of Virginia and staff physician at the Veteran's Administration Hospital, Richmond. Born in Zagreb, Yugoslavia, he received his M.D. degrees from the Medical School of the University Zagreb. After coming to the United States, he completed internship at the Salem Hospital in Salem, Massachusetts. Following that, Dr. Vlahcevic served on the faculties of Tufts and Western Reserve Medical Schools.
the “spasm reactor” in your practice
The Machine Age man still possesses a Stone Age stomach; sometimes the job of merely coping with today's environmental stress may prove too much. For some (the "spasm reactors" in your practice), tension, anxiety and worry may find expression through the voice of gastrointestinal or other smooth muscle spasm. To treat these patients with antispasmodics alone is often to miss the point of origin of their disturbance; to rely solely on tranquilizers often proves discouragingly slow or ineffective in relieving spasm and pain.

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Brief summary Blurring of vision, dry mouth, difficult urination, and flushing or dryness of the skin may occur on higher dosage levels, rarely on usual dosage. Administer with caution to patients with incipient glaucoma or urinary bladder neck obstruction. Contraindicated in acute glaucoma, advanced renal or hepatic disease or a hypersensitivity to any of the ingredients.

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<td>(% gr.) 48.6 mg.</td>
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A·H·ROBINS COMPANY, RICHMOND, VIRGINIA
Whenever anxiety induces or intensifies clinical symptoms

Librium®
(chlordiazepoxide HCl)

Quickly relieves anxiety—Helps improve response in psychophysiologic disorders—Seldom impairs mental acuity or physical coordination, on proper dosage—Has wide margin of safety

Before prescribing, please consult complete product information, a summary of which follows:

**Indications:** Indicated when anxiety, tension and apprehension are significant components of the clinical profile.

**Contraindications:** Patients with known hypersensitivity to the drug.

**Warnings:** Caution patients about possible combined effects with alcohol and other CNS depressants. As with all CNS-acting drugs, caution patients against hazardous occupations requiring complete mental alertness (e.g., operating machinery, driving). Though physical and psychological dependence have rarely been reported on recommended doses, use caution in administering to addiction-prone individuals or those who might increase dosage; withdrawal symptoms (including convulsions), following discontinuation of the drug and similar to those seen with barbiturates, have been reported. Use of any drug in pregnancy, lactation, or in women of childbearing age requires that its potential benefits be weighed against its possible hazards.

**Precautions:** In the elderly and debilitated, and in children over six, limit to smallest effective dosage (initially 10 mg or less per day) to preclude ataxia or oversedation, increasing gradually as needed and tolerated. Not recommended in children under six. Though generally not recommended, if combination therapy with other psychotropics seems indicated, carefully consider individual pharmacologic effects, particularly in use of potentiating drugs such as MAO inhibitors and phenothiazines. Observe usual precautions in presence of impaired renal or hepatic function. Paradoxical reactions (e.g., excitement, stimulation and acute rage) have been reported in psychiatric patients and hyperactive aggressive children. Employ usual precautions in treatment of anxiety states with evidence of impending depression; suicidal tendencies may be present and protective measures necessary. Variable effects on blood coagulation have been reported very rarely in patients receiving the drug and oral anticoagulants; causal relationship has not been established clinically.

**Adverse Reactions:** Drowsiness, ataxia and confusion may occur, especially in the elderly and debilitated. These are reversible in most instances by proper dosage adjustment, but are also occasionally observed at the lower dosage ranges. In a few instances syncope has been reported. Also encountered are isolated instances of skin eruptions, edema, minor menstrual irregularities, nausea and constipation, extrapyramidal symptoms, increased and decreased libido—all infrequent and generally controlled with dosage reduction; changes in EEG patterns (low-voltage fast activity) may appear during and after treatment; blood dyscrasias (including agranulocytosis), jaundice and hepatic dysfunction have been reported occasionally, making periodic blood counts and liver function tests advisable during protracted therapy.

**Usual Daily Dosage:** Individualize for maximum beneficial effects. Oral—Adults: Mild and moderate anxiety and tension, 5 or 10 mg t.i.d. or q.i.d.; severe states, 20 or 25 mg t.i.d. or q.i.d. Geriatric patients: 5 mg b.i.d. to q.i.d. (See Precautions.)

**Supplied:** Librium® (chlordiazepoxide HCl) Capsules, 5 mg, 10 mg and 25 mg—bottles of 50. Libritabs® (chlordiazepoxide) Tablets, 5 mg, 10 mg and 25 mg—bottles of 100. With respect to clinical activity, capsules and tablets are indistinguishable.

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