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Subject: Liver Function in Health and in Disease
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DR. CORDERO: As you know, the liver is the largest organ in the body and has a large number of functions so complex that it is difficult to point to any one particular function and say that it is the most important. Dr. Reissmann's lecture this morning will be the last seminar for the semester. I am sure that with his characteristic capacity for presenting a difficult subject in an easily comprehensible form, Dr. Reissmann will make this last lecture very interesting.

DR. REISSMANN: From an anatomical standpoint we can distinguish five functional systems in the liver: first, the parenchymal cells; secondly, the reticulo-endothelial cells; thirdly, the bile ducts; fourthly, the vascular system of the liver; and finally the lymphatics. The vascular system of the liver is rather peculiar and can stand some refreshing of the memory, I think. We must be aware that the liver receives the largest percentage of its blood from a vein, and the important clinical implication is that even the slightest obstruction of the blood flow thru the liver will result in portal hypertension. The lymphatics of the liver are also of considerable clinical importance because they play a considerable role in jaundice, and I will enlarge upon this subject a little later on.

The classification of the liver functions from a biochemical or physiological standpoint is difficult. In the first place, *in vivo* we are only able to measure a small percentage of the enormous number of metabolic functions of the liver, those anabolic as well as catabolic. Furthermore, we cannot measure these functions directly *in vivo*. We have to rely upon the appearance or disappearance of substances in the blood stream or in the body

excretions. The classification of the liver functions is furthermore made difficult by the different viewpoints clinicians, physiologists and biochemists have.

For instance, the clinician is mainly interested in measuring liver functions for diagnostic purposes and he asks at least three or four important questions: (1) Is liver disease present in a non-jaundiced patient, and if so, what is the extent of this liver disease? (2) In a jaundiced patient, he wants to know if he is dealing with a prehepatic jaundice (jaundice which is caused by an excessive breakdown of hemoglobin in the presence of a normal liver) or with an intrahepatic jaundice (jaundice caused by damage of the parenchymal cells of the liver) or with a post-hepatic jaundice caused by obstruction of the bile ducts. (3) In case of intrahepatic jaundice, he is furthermore interested to learn something about the extent of the liver damage. In other words, under acute conditions, he wants to know how many of the liver cells are being damaged at the time of the jaundice, and in chronic cases, he rather wants to know how many of the functioning liver cells are left. These are few of the problems the clinician is interested in.

Now, the physiologist and the biochemist are interested in other aspects. The physiologist wants to know the relationship of liver function to the functioning of other organs and the biochemist, for instance, is mainly interested in the intermediary metabolism which goes on in the liver cells. For these reasons, a single classification of liver function is almost impossible. On the other hand, there are about a hundred liver function tests available at least in the literature, and I think at least 10 or 12 of these liver function tests are commonly used in hospital practice. Even these 10 or 12 tests are rather confusing to the students, and we need some sort of a guide to be able to interpret these tests and to make sense out of the results obtained. Now, in order to do that, I propose to follow a simple classification of liver function; namely, (1) The removal of substances from the blood stream. These substances are either excreted in the bile or they are metabolized in the liver directly. Bilirubin is an example of the former while the breakdown of the steroids and other hormones in the liver is an example of the latter. (2) The adding of substances to the blood stream by the liver. This covers the synthesizing action of the liver, especially in relation

to the protein metabolism. (3) Finally, we have the storage function of the liver. Where liver cells disintegrate, storage compounds or integral compounds of the liver cells are released into the blood stream and as you will see later provide very important diagnostic proofs.

In accordance with this classification, I propose to discuss the more important liver function tests and as we go along, I will attempt to point out the information we can obtain from these function tests in the various types of liver disease. The removal function of the liver is, of course, exemplified best by the bile pigment. You all know that the bilirubin is derived from the breakdown of hemoglobin. At the end of the 120-day life of the normal red cells, the red cell disintegrates and the small chunks of hemoglobin are taken up by the reticuloendothelial system thruout the body. Within the cells of the reticuloendothelial system, the porphyrin ring of the hemoglobin is opened and the bile pigments are formed. From the reticuloendothelial system the bile pigments are sent to the liver for further excretion, and the important fact is that these bile pigments are still attached to a protein molecule. It was originally thought that this protein was a globin of the hemoglobin; this has recently been challenged. At any rate this bilirubin which is on its way from the reticuloendothelial system to the parenchymal cells of the liver is attached to a protein molecule and therefore constitutes a very large molecule. It is engulfed by the liver cells and pushed thru the liver cells into the bile ducts and during this process the protein molecule is removed. The bilirubin is then conjugated, and reaches the intestines as a conjugate.

For a long time, methods had been devised to distinguish between two types of bilirubin—the one where the protein molecule is still attached and the other which has no protein molecule. The van den Bergh's reaction was thought for a long time to be a quantitative measure of these two bilirubins in the sense that the indirect van den Bergh measures the bilirubin-globin and that the direct van der. Bergh measures the free bilirubin. That means the bilirubin that has passed thru the parenchymal cells of the liver. I understand you are using here a different method and you call the two bilirubins, the bilirubin-1 which is chloroform soluble and the bilirubin-2 which is water soluble. I

have no experience with this method but we suspect that the B2, the water soluble bilirubin of your method corresponds to a certain degree with the direct bilirubin as measured by van den Bergh's method. A considerable amount of confusion has been introduced in the question of these two bilirubins because it was thought that by measuring the direct and the indirect bilirubin, one would be able to distinguish intrahepatic from posthepatic jaundice. For reasons which we will discuss in a minute, this is obviously impossible. However, the distinction between these two bilirubins is a great help in elucidating the presence of prehepatic jaundice, that is, hemolytic jaundice, because at least semi-quantitatively the van den Bergh reaction can ascertain the presence of the bilirubin-globin, the indirect reacting bilirubin. And if you see a jaundiced patient and you find that most of the bilirubin in the blood stream if of the indirect type, then this is almost sufficient to make the diagnosis of prehepatic jaundice. Nature itself has provided a diagnostic test of this sort, because the bilirubin-globin being a very large molecule is not brought into the glomerular filtrate and consequently in prehepatic or hemolytic jaundice you will find very little bilirubin in the urine. This point is very frequently neglected although it should be the starting point in the differential diagnosis of jaundice; namely, the first thing the physician should do is to inspect the urine and if the urine is light in color but the patient is deeply jaundiced, then this is a very strong proof that you are dealing with a hemolytic type of jaundice. In some instances, of course, in hemolytic crisis, the urine is dark brown. This is not due to bilirubin but rather due to methemoglobin. Of course when the concentration of the hemoglobin in the blood plasma becomes very high, considerable amounts of hemoglobin appear in the urine and are usually converted into methemoglobin which is brown. By performing a simple test of bilirubin in the urine, we can distinguish between methemoglobin and the presence of bilirubin.

Now, in intrahepatic and posthepatic jaundice, the bilirubin is mostly of the direct type. In order to understand this, we have to discuss briefly the mechanism by which the jaundice is produced in hepatitis or in intrahepatic jaundice. It is frequently thought that the jaundice in intrahepatic damage is due to the fact that the liver cells are unable to remove the bilirubin-

globin from the blood stream and excrete in into the bile. This is not so, and a simple experiment can show us the reason why. We can remove considerable parts of the liver in experimental animals. The rat is particularly suitable because we can clearly distinguish 6 or 7 discrete lobes of the liver and they are very easy to remove. We can remove 50 per cent of the liver tissue rather easily. Under these circumstances no jaundice develops. Leaving only 50 per cent of the parenchymal tissue of the liver intact, this 50 per cent of the liver cells are completely able to handle the excretion of bilirubin. In other words, we have a rather large margin of safety as far as the number of liver cells are concerned. On the other hand when we produce intrahepatic damage, for instance, by feeding carbon tetrachloride to a dog, we will already see the appearance of jaundice after 20 per cent of the liver cells are damaged although the remaining 80 per cent of liver cells are functioning in a fairly normal manner. Now, what is the reason for this difference? The reason is that when we have damage within the liver as in the case of carbon tetrachloride poisoning, the other liver cells grab the bilirubin globin and excrete in into the bile ducts but from the bile ducts it passes back into the blood stream because the barrier between the bile ducts and the blood stream on the one hand and between the bile ducts and the lymphatic system on the other hand is broken and therefore the bile that is normally secreted into the bile ducts passes back either directly into the blood stream or via the lymphatics into the blood stream. And this is the reason why in cases of intrahepatic jaundice, we find predominantly the direct reacting bilirubin in the blood stream.

The same findings are obtained in posthepatic jaundice, in surgical jaundice due to an obstruction of the bile ducts, because there we have pretty much the same mechanism. The bilirubin is grabbed by the parenchymal cells of the liver, the protein is detached, the bilirubin is secreted into the bile ducts but due to the obstructions, it regurgitates into the blood stream, and in obstructive jaundice most of these regurgitations go via the lymphatics. It is a mistaken idea to think that the regurgitation occurs directly from the bile ducts into the sinusoids of the liver. That is only the case later on in the game when larger portions of the liver become necrotic as a result of the bile obstruction. During the first two weeks or so of an obstructive jaundice,

most of the bilirubin reaches the blood stream via the lymphatics of the liver. And when you ligate the bile ducts in a dog, you will see after one or two weeks a very large extension of all the lymphatics coming out of the liver; and these lymphatics are full of bilirubin and carry the bilirubin via the thoracic duct into the circulation. From this discussion of the mechanism of the jaundice in these two conditions, it is obvious that no bilirubin measurement can give us the distinction between these two types of jaundice. Thus, in intrahepatic as well as in surgical jaundice, the circulating bilirubin is primarily the B2 bilirubin. So we have to look for other liver function tests to make a differential diagnosis in these two conditions.

In order to proceed in a systematic way, we better discuss the other substances that are excreted in the bile. We have cholesterol which is likewise used as an important index of liver function. Cholesterol is synthesized to a very large extent in the liver and this cholesterol, a major portion of this synthesized cholesterol, is excreted in the bile, reaches the intestines and unfortunately is reabsorbed in the intestines. That of course is the difficulty of those investigators who are engaged in finding measures to produce a negative cholesterol balance in order to cure or prevent atherosclerosis. Now, it was found that this excretion of cholesterol is mainly disturbed in obstructive jaundice. That means when we have an obstruction of the bile ducts, the synthesis of cholesterol in the liver goes on but this cholesterol is secreted into the blood stream and gives rise to very high blood cholesterol. The high blood cholesterol is one of the significant findings in obstruction of the bile ducts. On the other hand, when have an intrahepatic damage, hepatitis, then the cholesterol synthesis is diminished. Therefore in intrahepatic jaundice the total cholesterol usually remains normal or is even below normal.

The alkaline phosphatase is likewise excreted in the bile and it is also an important diagnostic test for obstructive jaundice. Alkaline phosphatase is an enzyme which splits phosphate groups from organic phosphorus compounds, and it is called the alkaline phosphatase because it has its maximum activity in alkaline medium. There are several sources of alkaline phosphatase. It is produced in the bone, of course; it is probably produced in the intestinal mucosa and carried by the flow of

blood, and it is furthermore produced to a certain extent in the liver. But all these alkaline phosphatases are excreted by the liver in the bile. And when we have an obstructive jaundice, the excretion is of course impaired and we have an increase of alkaline phosphatase in the blood stream.

There are several other conditions, however, which lead to an increase of alkaline phosphatase. Neoplastic disease of the liver is one, particularly hepatoma. Very high alkaline phosphatase levels in the blood have been reported and this takes place in almost 90 per cent of all the hepatomas. Therefore, a very high alkaline phosphatase which is out of proportion to the jaundice always suggests a malignancy of the liver, especially a hepatoma. The reason why the alkaline phosphatase is very high in hepatoma is not quite clear. Some investigators think that the neoplastic cells of the hepatoma secrete some alkaline phosphatase. So with the total cholesterol and the alkaline phosphatase, we already have two liver function tests which are highly significant in obstructive jaundice and specially in the discrimination of obstructive jaundice from intrahepatic jaundice.

Now, let's turn to the other group of liver functions, namely, the synthesizing function of the liver. We all know that most of the proteins, perhaps with the exception of some globulins of the plasma protein, are synthesized in the liver, and therefore the protein levels in the blood plasma can be used as a fairly reliable test of the liver function under certain circumstances. We have to remember that the life span of the protein molecules varies a great deal. For instance, an albumin molecule stays in the circulation for at least three weeks, and the life span of the globulin molecule is approximately the same. In other words, when there is considerable damage to the parenchymal cells of the liver and these liver cells are unable to synthesize any albumin at all, then it still will take at least two weeks before the albumin level in the plasma would fall by 50 per cent. This is frequently forgotten and the measurement of the albumin concentration in the blood plasma is carried on in cases of acute hepatitis where the time element is such that we possibly cannot find any changes in the albumin concentration at that time.

On the other hand, the life span of the prothrombin, also of course a protein which is synthesized by the liver, is much shorter. For some obscure reason, the life span of the prothrombin molecule is in the order of 2 days, 3 days perhaps at the most. You know that from the use of dicumarol, of course. When you give large amounts of dicumarol and inhibit the synthesis of prothrombin completely, then within 24 or 36 hours we find a very considerable drop of the prothrombin concentration in the blood plasma. And if one remembers that, then one can clearly understand that the prothrombin level is a test that becomes positive faster in acute hepatocellular damage. In other words, when we have a patient with hepatitis with jaundice for 2 or 3 days, then it is almost senseless to do an albumin determination in the blood plasma because you know it takes at least 2 or 3 weeks before we can expect any changes there. But if we do a determination of the prothrombin then we can see a significant decrease in the prothrombin in 24 to 48 hours after the damage.

A decrease in the prothrombin is also produced, of course, by obstructive jaundice because the absorption of Vitamin K requires bile salts, and this gives rise to the test where we inject Vitamin K subcutaneously and see whether we find any increase in the prothrombin content of the blood plasma. Such a test obviously permits the discrimination between obstructive jaundice and hepatocellular jaundice. When the liver cells are damaged then the injection of Vitamin K will not increase the prothrombin, because the factories are destroyed and regardless of how much of the raw materials is offered they cannot produce. On the other hand, when we have obstructive jaundice and the liver cells are still in reasonable shape but cannot produce prothrombin because they do not have Vitamin K then by the subcutaneous administration of Vitamin K we can, of course, enhance the formation of prothrombin. And this is also a test which is of great importance in the distinction of hepatocellular *versus* surgical jaundice. Now, if the jaundice persists, however, for a longer period of time then the albumin measurement becomes very important; and also in patients who have a silent cirrhosis. In other words, patients who are only slightly jaundiced but in whom a great amount of liver cells were destroyed earlier, in these patients, I think, the measurement of the al-

bumin concentration is the best liver function test as far as the overall liver function is concerned. In other words, one may estimate just from the level of the albumin approximately how many functioning liver cells are still intact in a particular patient.

Another test which is frequently employed and which actually belongs under the heading secretion or excretion test is the bromsulfophthalein, the BSP, and that is a test which is based upon the fact that the BSP is selectively excreted by the liver. Some of it is destroyed by the liver but most of it is excreted into the bile. The BSP is, in my opinion, one of the most sensitive liver function tests. That means if you have a patient who has an enlarged liver but who has no jaundice and you would like to find out whether this patient has any liver damage then the test you should use is the BSP test. Because if the BSP test is normal then you can definitely say that this man has no liver damage whatsoever. If a patient, however, is jaundiced then the BSP test is completely unnecessary. It is frequently a misconception that it is impossible to do the BSP test in a jaundiced patient because they are unable to measure the blue dye in the blood plasma in the presence of bilirubin. This, of course, is not true because the light absorption of the blue dye BSP occurs in an entirely different aspect or region from that of bilirubin, and by means of a photo-electric colorimeter you have no difficulty whatsoever in measuring the blue dye in jaundiced patients. But it is senseless to do the test because the jaundice tells us that the patient has some liver disease and an abnormal BSP test simply tells us just the very same thing. In other words, an abnormal BSP test signifies that there is some disease in the liver, in the blood vessels of the liver or blood supply of the liver or bile ducts, but it does not enable us to distinguish well this defect or where this disturbance is located. Therefore, I would like to emphasize again the BSP test is a wonderful test in the non-jaundiced patient when you want to know if there is liver damage or not. In all other kinds of liver diseases, the BSP test cannot give us any valuable information at all. So as far as the metabolic function is concerned in acute cases, I think you should use the prothrombin and in chronic cases you should rely on the albumin measurement.

Another group of tests which is also related to protein metabolism are the flocculation tests and there are at least 20 or 30 different flocculation tests. All these tests are based upon the fact that the colloid stability of the blood plasma depends on 2 factors: the presence of albumin which makes it stable and the presence of gamma globulins which makes it unstable. In other words, when we have an increase of gamma globulins then the blood plasma in contact with some colloidal solutions will decrease the stability of this colloidal solution and produce some flocculation. On the other hand, when we have a plasma with very low albumin, the same thing will occur. You have the combination of the two, a lower albumin plus a high gamma globulin. Now, if you realize these very simple facts then you know that the flocculation tests are by no means specific for any liver disease. In any occasion where you have an increase of gamma globulin, for instance, in chronic infection, in rheumatism, things like that, the flocculation tests are very frequently abnormal due to the high concentration of gamma globulin in the plasma of the patient. Likewise in cases where the albumin is abnormally low as it is in a case of nephrosis, for instance, the flocculation test may because abnormal.

The increase in gamma globulin in acute liver diseases like hepatitis or in chronic liver diseases, like cirrhosis, is thought to be due to the irritation of the reticulo-endothelial system in the liver. This, of course, is one of the times when we explain or try to explain a fact simply by using a word because I have left it to your imagination whatever irritation of the reticulo-endothelial cells means, and so I think it is better if we say that whenever we have a disease inside the liver that affects the reticulo-endothelial system then the flocculation test may be abnormal. Thus, their application lies: (1) in acute hepatitis and (2) in cirrhosis, specially in Laennec's cirrhosis.

Now the third group, and I have to rush a little because our time is running out, the third group of liver function which we mentioned are related to the storage function of the liver. One of the substances that is stored in the parenchymal cells of the liver is iron. Of the 5 grams of total iron we have in our body about one gram or so is present in the parenchymal cells of the

liver and it is present there combined with a very specific protein. Now, it has been observed that in cases of acute hepatitis, the serum iron becomes considerably increased. That means, the iron that circulates in the blood plasma or in combination rather with a globulin, increases from a normal of 100 mg. per 100 ml. to, say, 200 to 300 mg. per 100 ml. It was thought for a long while that this was a manifestation of the disturbance of the storage function of the liver in relation to the iron metabolism. But it was shown later on that this was not so and that this serum iron is due to a very simple effect, namely, when liver cells are damaged then the stored iron leaks out and is found in the blood plasma. Likewise, when liver cells become necrotic, the iron comes out, and of course, it goes into the blood plasma and circulates there for a couple of days. In this way we could advance the concept that an acute increase in serum iron as seen in acute hepatitis is a direct reflection of the disintegration of liver cells. This is rather an important concept because all the other liver function test measure more or less the function of the surviving liver cells. In other words, many of these tests depend on how many of the liver cells survive and are still functioning but if you find, for instance, there are only 50 per cent of the liver cells surviving, you may not know at what time the other 50 per cent were destroyed. It might have been destroyed 2 years ago or it might have been destroyed 2 days ago. Now, with this test and the serum iron is one of the best of these tests, you can directly obtain evidence of how many liver cells are disintegrating within a very short time before or during the test. The disadvantage of the serum iron of course is the fact that in many people, the liver does not contain very much iron. If a person has an iron deficiency, to start with, then there is practically no iron in the liver and if the liver cells of such person disintegrates, you cannot expect any serum iron increase. The other disadvantage is that other conditions like hemochromatosis or hemolysis, things like that, likewise result in an increase of the serum iron. It was therefore a distinct advancement when transaminase measurement was proposed as an indicator of acute liver damage. The transaminase is an enzyme which is present in many cells of our body and which is concerned in shifting amino groups therein, and according to the substrate and to the receiver of the amino groups, we have several different transaminases. The one

that is most commonly used for diagnostic purposes is the glutamic oxaloacetic transaminase. That means the glutamic acid is the substrate and the oxaloacetic acid the receiver of the amino group and the enzyme that does it is the GO transaminase. Now, the transaminase that is present in the blood stream is rather low in concentration, we find perhaps 20 units, while it is present in the liver cells and the cells of the heart muscle in very high concentrations. For instance, we find concentrations of 200 to 200,000 units per ml. and as in the case of serum iron whenever liver cells disintegrate then the transaminase leaves these liver cells, go into the blood stream, circulates there for one or two days and can be measured. This is a tremendous advancement, I think, because we now have tests which directly tell us about liver cell disintegration the same day or may be within 48 hours when the test is carried out. Furthermore, the magnitude of the enzyme level under these circumstances gives us some estimation of the degree of liver damage. That means that if we have only a moderate elevation of the transaminase, we can conclude that only a few liver cells, perhaps 5 per cent or so of the liver parenchyma, are being damaged. If you have very high transaminase — 3,000, 4,000 or 5,000 units — that indicates that a large percentage of the liver cells has been damaged and that therefore the prognosis is much graver.

These are the principal liver function tests that I wanted to discuss today and if we summarize it from another standpoint, from another viewpoint, then we can say that in prehepatic jaundice, that means in hemolytic jaundice, the diagnosis is based upon high indirect bilirubin in the blood stream, the absence of bilirubin in the urine and upon a few hematological tests like the reticulocyte count. In intrahepatic jaundice, we have to distinguish the acute stage when liver cells are disintegrating and during the acute stage, the transaminase is by far the best proof. During chronic stages, that means after the liver cells had disintegrated then the tests that are related to the synthesizing function of the liver are of prime importance and it is specially the serum albumin and the percent of cholesterol ester that give reliable information. In surgical jaundice, obstructive jaundice, we make use of alkaline phosphatase and cholesterol. Furthermore, we have the possibility now to rule

out an acute intrahepatic damage by means of the transaminase and the serum iron determinations. In this way, I think we will have no difficulties in making our differential diagnosis.