HEPATIC EFFICIENCY TESTS

with a report on

THE GALACTOSE TOLERANCE TEST.

by

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INTRODUCTION.

The last quarter of a century has witnessed many advances in medicine, and not the least of these has been the application of biochemical and biophysical methods in the investigation of disease. The success which has attended these efforts, is probably best seen in the discovery of Insulin, but other advances perhaps not so epoch-making, have been made, and it is impossible at present to foretell to what heights they will lead us, or whether they will ultimately assist at all in the elucidation of the numerous problems awaiting solution.

These methods have resulted in the evolution of so-called "function tests" applied to various organs of the body, and in spite of numerous theoretical objections which can be lodged against them, and they are many, there can be no doubt that they have yielded results which have proved useful both in diagnosis and prognosis though the ultimate interpretation of these results may be a matter of dispute.

In the following paper the writer is concerned with the

liver, the various tests which have been evolved for estimating whether disease be present, and if so, to what extent, and their value in the prognosis of disease.

Various difficulties, which, though mainly theoretical, affect the practical value of conclusions, are met with at the outset.

1. An organ has usually many functions, while the tests applied, usually measure but one of these.

2. Deficiency in the particular function investigated does not necessarily imply deficiency in all, or even in any other function.

3. The large reserve power possessed by most organs of the body. This is well seen in the case of the liver. Up to three fourths of liver substance may be removed, without affecting appreciably the bodily economy.

4. The powers of hypertrophy and hyperplasia possessed by the liver and the ability thus to compensate for the effects of disease.

5. The particular function tested may be possessed by other organs to a varying extent, and so deficiency in an organ may be compensated by over-activity in another.

6. Each chemical process is but a part in the total metabolism

of the body, and failure in one link of the chain of reactions will react on all, so that it is necessary to rule out coincident disease in other organs.

Finally the question arises - what is the position when a laboratory finding is at variance with a clinical one? This can only arise from a mistaken conception as to the relative value of clinical and laboratory data.

There is no essential difference between estimating the capacity of the heart for doing work and assessing the power of the kidney to excrete urea. Each item is but one link in the diagnostic chain, and must be considered in relation to the whole.

The task of interpretation of results is therefore very great, and caution necessary before definite conclusions can be drawn.

THE EXPERIMENTAL BASIS OF HEPATIC INSUFFICIENCY.

In order to determine the effects of insufficiency of any gland, the simplest method is to remove the gland, partially or in toto. In the case of the liver of mammifers, however, a preliminary anastomosis between the portal vein and vena cava must be made by means of an Eck fistula, otherwise the blood accumulates in the portal system, and the animal dies from the resultant circulatory disturbance. Under these conditions, the phenomena observed after removal of the liver can be legitimately ascribed to absence of the organ. It must be borne in mind, however, that complete hepatectomy is a brutal operation, and that inside a few minutes an abnormal condition is established which normally takes weeks or months for pathological processes to accomplish; the duration of survival after the operation is brief, and too short to allow other organs to take over the functions of the liver; finally, each species of animal has its own peculiar metabolism, which must be borne in mind before drawing conclusions in man.

In an attempt to approximate closer to pathological conditions

chemical substances having an elective affinity for the liver have been introduced into the body, or even injected into the bile-ducts, with the object of causing destruction of the liver. The results obtained by these methods cannot be compared with those following hepatectomy. This can be readily understood, since some of the cells always escape destruction by the toxins, while those affected remain in situ, and liberate injurious substances which can affect the rest of the organism, so that, to the symptoms depending on functional deficiency, are added those of poisoning by autolytic products.

Effects of Removal of the liver in various animals:

In frogs, removal of the liver can be performed directly, on account of the porto-caval anastomosis which is normally present in the frog-family as well as birds. The period of survival is from 3 to 7 days if the animal is placed in water which is not kept fresh; in running water, the survival is about 3 months. After the operation, the urine no longer contains urea.

In birds, in whom the operation can be performed without any preliminaries on account of the existence of the vein of

Jacobson, death ensues in about twenty hours; during this period, there is complete anuria in the pigeon; in geese, urine is secreted, but this becomes clear and acid, instead of turbid and alkaline as it normally is. Uric acid, which in the goose forms 60% to 70% of the total nitrogen excreted, falls to 6% or even 3% after hepatectomy. If, now, these geese are fed on urea or amino-acids, these substances are recovered unchanged in the urine, while in the normal animal, they are eliminated as uric acid. Ammonia, which normally forms 9% to 18% of the total urinary nitrogen, amounts to more than 60% after removal of the liver, and ammonium lactate accummulates in the organism. These results illustrate the important part played by the liver in nitrogenous metabolism. Neither bile pigments nor bile acids accumulate in the blood, which is contrary to what one would expect if the site of production was outside the liver, and one cannot plead that the time of survival is too short for this to occur, since, after ligature of the bile duct, biliary pigments accumulate in the blood after 6 hours.

The animal dies in a state of collapse, sometimes preceded by convulsions.

Most of the modern experimental work on hepatectomy has been done on the dog. The first step consists in making an Eck fistula, i.e. establishing an anastomosis between the portal vein and the vena cava. Perroncito then waits several months before performing the second operation, and does not remove the liver till the animal has completely recovered; the organ which is removed however, is not a normal liver, but one in which the circulation has been reduced. Mann and McGath operate in three stages; the first stage consists in making an Eck fistula, and ligaturing, not the portal vein, but the vena cava below the porto-caval anastomosis and in front of the sub-hepatic veins; very rapidly, the peripheral anastomoses are established, sometimes uniformly, sometimes by the predominance of one or other group of veins. In the second stage operation, performed at least one month after the first, the portal vein is tied close to the liver; this can now be performed without danger; then, after another interval of a month, the hepatic artery and the inferior vena cava at the level of the diaphragm are ligatured, and the liver is removed. In this case also, the liver has been affected, at first by an increase in the circulation, and finally by a great decrease. These alterations in the blood-supply

to the liver, however, are unavoidable, since the blood must be returned to the general circulation, and the two operations cannot be performed simultaneously.

The carbohydrate and nitrogenous metabolism are affected. Mann & McGath have shown the importance of hypoglycaemia. After a latent period, which lasts from 2 to 8 hours, the first symptoms appear; these consist of progressive muscular asthenia; the animal with difficulty keeps its legs; he lies down on the ground; in less than an hour, he is unable to perform the slightest movement; but this flaccid condition of coma does not last long. Soon, motor hyperexcitability is noticed, which at first is localised. A sharp noise provokes a brisk contraction of the legs; muscular twitchings appear, at first affecting a single muscle, then a group of muscles. Finally, the slightest stimulus causes generalised convulsions; nausea and vomiting supervene, and the animal succumbs. The rate of the heart, which is increased after the operation, becomes slowed during the asthenic phase. The arterial pressure decreases from the onset of symptoms. The respirations, which are rapid during the post-operative period, towards the end assume the Cheyne-Stokes rhythm. The temperature remains normal till the stage of coma; it may even rise one degree before the asthenic

phenomena appear; then the temperature falls during the comatose period, but the fall is moderate.

Most of these symptoms are due to hypoglycaemia, and can be promptly removed by the administration of glucose. The blood-sugar which has already been lowered by the Eck-fistula, becomes progressively less. Symptoms commence when the blood-sugar reaches 0.5 gm. per litre. The fall becomes more rapid when convulsions commence, and death takes place when the blood-sugar is about 0.30 gm. per litre. What proves unmistakably, that the sugar-deficiency is the cause of the symptoms is that the intravenous injection of 25 - 50 cg. of glucose per kilo. weight immediately revives the comatose dog; within 30 seconds, the animal stands up, walks about and drinks; in less than a minute he appears to become normal again; the arterial pressure regains its former level; the heart rate becomes regular; the respiration resumes its usual rhythm; the temperature becomes normal; the respiratory quotient and the basal metabolism are raised. If the animal had reached the convulsive stage, the results are even more striking; so long as the heart continues to beat, the animal can be revived. This remission, however, is temporary; its duration varies with several factors, whether the animal is

stimulated or kept quiet, whether or not it is kept warm, etc. Soon, the symptoms recommence; they are alleviated again by another injection. The animal can in this way be revived several times, but the injections require each time to be increased. Soon, new symptoms make their appearance, which the injection of glucose does not alleviate; sometimes they consist of coma with convulsions which rapidly leads to death, sometimes of disordered movements with absence of vision and hearing, quickly followed by coma and death. These symptoms are due to a profound disturbance of nitrogenous metabolism. This is confirmed by analysis of the urine, which shows a great increase in the ammonia nitrogen, which may reach 50% of the total nitrogen, an increase in the amino-acids, the appearance of uric acid which is usually absent in the urine of normal dogs, diminution in the blood urea which may fall to 0.017%, and an increase in the residual nitrogen.

The period of survival without glucose is from 6 to 7 hours; with intravenous injections of glucose, 19 to 20 hours; one dog, operated upon by Mann & McGath, survived 34 hours, by combining the various methods of introducing glucose. Thus, the early symptoms following hepatectomy are due to hypoglycaemia, and not to

poisoning by ammonia bodies, as was formerly held by Pawlow and Nencki. The glucose acts specifically, and not as a regulator of the Ph, which only undergoes slight modifications. It is of no use merely to maintain the osmotic power of the plasma; the administration of substances of even higher osmotic power than glucose is valueless. Neither other sugars nor physiological substitutes, nor extracts of glands causing hyperglycaemia, nor protein compounds containing a carbohydrate group, can replace glucose. The decrease in blood-sugar following hepatectomy is a generally observed phenomenon; it occurs in geese, in whom, however, the hypoglycaemia is not so marked as in dogs; it is also observed in the fish, tortoise and frog. Thus, one can conclude that removal of the liver deprives the organism of the greater portion of its carbohydrate reserve, and removes the chief seat of origin of glucose. The utilisation of the glycogen in the muscles, although this represents 50% of the total glycogen of the body, occurs too slowly to meet the needs of the tissues.

The action of the pancreas on carbohydrates is antagonistic to that of the liver. Removal of the pancreas causes hyperglycaemia and diabetes, while the hypoglycaemia which follows hepatectomy, is similar to insulin hypoglycaemia. If the liver is removed

24 to 48 hours after removal of the pancreas, the hyperglycaemia falls rapidly from 2 to 4 times the normal level, and symptoms appear at a blood-sugar concentration not so low as after hepatectomy alone; the injection of glucose again has a favourable affect, but its action is of short duration.

Thus, the liver plays a very important part in the regulation of the blood-sugar concentration. Its presence is necessary for the production of pancreatic diabetes; its removal causes the latter to disappear in a few hours. Similarly, asphyxial states, and the injection of adrenalin no longer cause hyperglycaemia when the liver has been removed. The pancreas, however, plays a part in the onset of symptoms following hepatectomy, and the absence of its internal secretion limits the utilisation of glucose. The longer hepatectomy is delayed, the higher is the blood-sugar at which symptoms appear, the last remains of the internal secretion of the pancreas still in circulation after removal of the organ having by then disappeared; glucose is then ineffective and cannot be utilised.

The changes in nitrogen metabolism after hepatectomy were formerly those first studied; Perroncito (8) concludes from his own experiments that after

extirpation of the liver, there is a diminution in urea with increase in ammonia and uric acid, but no acidosis is present, and death is not due to acid intoxication. Mann & McGath's conclusions (I) are as follows. The production of urea ceases after removal of the liver. In fact, from the onset of the secretion of urine, after hepatectomy, there is a marked diminution, not only in the urea of the blood, but also in the amount eliminated in the urine, as well as in the urea of the tissues especially the muscles. If the kidneys are removed at the same time as the liver, so that the animal becomes anuric, the urea of the blood remains the same, or else falls slightly. The slight diminution sometimes noted may be explained by the excretion of a certain amount of urea in the saliva and vomitus. If the kidneys are removed 8 to 24 hours before removal of the liver, by which time the blood urea is well above normal, no increase in the blood-nitrogen occurs.

The action of the liver on uric acid is also well demonstrated; normally, only traces of uric acid are found in the blood of the dog, and no appreciable amount appears in the urine. After removal of the liver, uric acid appears in the blood, and simultaneously, a large amount is excreted in the

urine, crystals being deposited immediately after being voided. Uric acid injected into the normal dog is rapidly destroyed; if injected into a hepatectomised dog, it is almost completely eliminated in the urine; if the kidneys are removed, the uric acid is found in the blood and muscles. These experiments prove conclusively that in the normal dog, uric acid is destroyed in the liver; from which one may conclude that in man the liver plays an important part in purine metabolism.

Removal of the liver is followed by a progressive increase in the amino-acid content of the blood, and no one has ever been able to prove the formation of urea after the injection of amino-acids in hepatectomised dogs. It appears, therefore, that utilisation of amino-acids is impossible after removal of the liver.

Effects of Partial Removal of the Liver:

Three-quarters of the liver of the dog or rabbit can be removed without the animal succumbing; after the second day, feeding takes place as before. The biliary secretion, which is greatly reduced in the first two or three hours following the operation, soon resumes its normal course. The urea and nitrogen index of the blood are diminished, while the residual nitrogen is increased.

The diminution in urea is proportional to the amount of parenchyma removed, the greatest fall observed following total removal. After partial resections, as Ponfick showed in 1889, the organ rapidly regenerates, and coincidently, the urea increases, and the nitrogen index tends to become normal. Within two or three months, the liver regains its former volume and weight.

Mann and McGath have noted that regeneration is delayed, if not completely suppressed, in animals having an Eck-fistula. If only the small median lobes remain, incomplete and permanent hepatic insufficiency results; the dog wastes, the appetite is lost and he becomes weaker, without becoming jaundiced. The blood-sugar is lowered; uric acid appears in the urine, and its increase is proportional to the intensity of the symptoms. Cure may occur after a long period; then the uric acid disappears from the urine, and at autopsy, a certain amount of regeneration is found.

According to Maddius, partial removal of the liver confers globulicidal properties on the serum.

Condition of the various hepatic functions after removal of the Liver:

The glycogen function, by virtue of which the liver is the great reservoir of glycogen in the organism, is suppressed, and this causes the rapid fall in the blood-sugar. This function is not confined to the liver; glycogen is still present in the muscles; but, as in that situation it constitutes a local reserve, and not a general or somatic reserve like that of the liver, it is incapable of replacing it. Nevertheless, Mann and McGath who estimated the glycogen in two portions of muscle, one portion removed during the operation and the other in the dying phase, found a diminution of 50%; a certain amount of glucose derived from the muscles can therefore be utilised by the hepatectomised animal.

The ureogenic function is almost completely abolished, since this function is practically confined to the liver, and the amount of urea which can be formed outside the liver is very small. In order to estimate the condition of this function, note must be taken of the nitrogen of the blood and urine; since if the kidneys are not functioning normally, the fall in blood nitrogen may be masked by the retention of urea due to renal failure. Since urea is no longer formed, amino-acids are excreted as such in the urine.

The uricolytic function is suppressed, so that uric acid increases in the blood and urine.

The action of the liver on the coagulability of the blood is not clarified by hepatectomy experiments. Most authors (Gley and Pachon, Hedon and Delezenne, Nencki and Pavlow, Salaskin and Zaleski; Mann and McGath), found no appreciable difference in the coagulability of the blood; Mann and McGath found that the fibrinogen content of the blood underwent no change. However, Doyon and Kareff found the blood became incoagulable after removal of the liver in the dog. Nolf has explained these apparently contradictory findings. The blood is coagulable after hepatectomy so long as its fibrinogen content does not vary; but if, through any cause, the blood loses its circulating fibrinogen, it becomes and remains incoagulable, since the liver is the only organ which produces fibrinogen. That is what happens if an intravenous injection of Witte's peptone is given, which causes coagulation of fibrinogen on the endothelium of the capillaries. That is what happened in the experiment of Doyon and Kareff; there the thromboplastic agent was the internal surface of the rubber tube connecting the portal vein to one of the sub-hepatic veins (2).

The biliary secretion is completely abolished by removal of the liver. If, as some authors hold, the liver only eliminates the pigments and salts formed outside the organ, these substances should accumulate in the blood when their path of excretion is removed. Now, nothing of the sort occurs; in the frog which sometimes survives three months after operation, and in the goose which succumbs in about twenty hours, there has never been demonstrated either icterus or hyperbilirubinaemia. In the dog, Perroncito has never found bilirubin either in the blood or urine. However, Mann states that he found in all hepatectomised animals surviving more than 6 hours, a yellow colouration of the urine manifest in the first specimen passed after the operation, and a similar colouration of the plasma, becoming deeper till death occurs, and a yellow tint in the sclerotics and sometimes in the mucous membranes of dogs surviving more than 16 hours; at autopsy, all the adipose tissue had a dark yellow colour. According to Mann, this pigment colouring the urine, tissues and blood is bilirubin.

These facts noted by Mann have been confirmed by other experimentors who operated by his methods. But, as Perroncito says (3) this yellow pigment has only been found by those authors

who injected intravenous glucose into their hepatectomised dogs, since these animals were deprived of the most important organ of carbohydrate metabolism. Moreover, not all authors who found this pigment admit that it is bilirubin; thus Melchiort, Rosenthal and Licht state that the pigment does not give the reactions of bilirubin, and that its formation is not influenced by conditions which normally cause an increased production of bilirubin. In conclusion, it may be said, that the possibility of the organism forming bilirubin outside of the liver cannot be denied, and this does not diminish the value of those experiments which prove that the liver is the chief seat of formation of its pigment and biliary salts. The same holds good for bilirubin as for glycogen and urea; the liver is not the only tissue capable of producing it, but the amount formed outside of it is very small compared with what it can produce.

Three Argentine authors, Saravia, Mazzocco and Royer, have estimated the biliary acids in the blood after hepatectomy, by the method of Aldrich and Bledsoe; they found that their content in the blood kept on increasing. They then inquired whether this increase was not due to reabsorption of the acids from the intestines. To answer this question, they made a biliary fistula

in dogs 5 to 7 days before performing hepatectomy, so as to divert the bile from the intestine; then they repeatedly purged the animals. These animals showed only a very slight increase in the biliary salts of the blood and in certain animals there was even a diminution; accummulation of biliary salts in the blood after hepatectomy only occurs when the intestine contains the salts and these when absorbed by the duodenal mucosa find the bile passages blocked and cannot be excreted into the urine(4). Similarly with urobilin, according to Royer and Saravia, who only found the latter in the blood after hepatectomy when it was present in the intestines; it accummulates in the blood because the kidney will not excrete it; so that urobilinaemia increases after hepatectomy while urobilinuria does not vary even after the intravenous injection of urobilin (5). Thus, one may say that the production of the chief constituents of bile, pigments and salts, ceases after removal of the liver; if small quantities are still formed under certain circumstances, these have no connection with what is produced by the normal activity of the liver; since the occurrence of jaundice presupposes the existence of the biliary secretion, one can conclude that not only is it not a sign of hepatic insufficiency, but its non-appearance is due

to deficiency of the gland.

Effects of Destruction of the Liver by Chemical Compounds:

Denys and Stubbe, on injecting a 2 - 5% solution of acetic acid into the biliary tract of the dog, caused destruction of the liver parenchyma without affecting the circulation in any way (6). They state that the symptoms are identical with those observed by Minkowski, following removal of the liver in the goose; convulsions, at first localised, then general, then death of the animal in 6 to 26 hours. Urea is greatly diminished in the urine secreted after the operation; the temperature is subnormal, the rectal temperature falling to 33.7° and even to 32.5°. In the rabbit similarly treated, the temperature falls even lower, and may reach 27°; similar results were obtained by Roger. These researches are very interesting, and should be reviewed in the light of recent knowledge, concerning the blood-sugar concentration, the blood nitrogen, the urea and amino-acids of the urine, and fibrinogen, to see whether the effects of chemical destruction of the liver, and operative removal are analogous. One essential difference is that hepatectomy hardly lowers the temperature, contrary to what occurs after the operation

of Denys and Stubbe, and, in considering the question, Roger thinks that the great lowering of temperature which he found is due to abnormal function of the gland, and not to suppression of hepatic function.

Icterus was never noticed in Denys and Stubbe's cases. However, the one animal which survived the operation, developed, a few days later, a slight yellow tint of the sclerotics. It was then killed, when the liver and serum were found to have an icteric colour, while the urine gave a markedly positive Gmelin's reaction. At this stage, the animal was convalescent, and the jaundice, far from corresponding to a phase of hepatic insufficiency, coincided with the period of regeneration of the liver, which showed manifest hypertrophy.

Certain substances injected subcutaneously, or introduced by the digestive tract, cause extensive damage to the liver. In phosphorus poisoning, some of the symptoms resemble those following removal of the liver, such as decrease of urea in the urine, which follows a brief period when the urea is increased. But Mann and Williamson, who studied experimentally the effects of phosphorus and chloroform poisoning in the dog, noted the absence of symptoms usually ascribed to removal of the liver, and particularly, hypoglycaemia, which if not always absent, was

always of only slight degree. They found however, increase in uric acid and amino-acids, as well as diminution of urea. They concluded that the hepatic element, while it explained some of the symptoms, was incapable of accounting for all the phenomena following these intoxications.

To summarise the foregoing, absence of the liver, whether due to partial or complete experimental removal, is followed by:

1. hypoglycaemia, the blood-sugar not being supplied as fast as it is used up;

2. diminution in the nitrogen of the blood and urine, due to cessation of formation of urea, which is almost all formed by the liver.

3. increase in amino-acids of the urine, the amino-acids not being transformed into urea, and eliminated unchanged;

4. increase in uric acid of the urine, uric acid not being destroyed as it normally is, in the dog;

5. cessation of formation of fibrinogen, which only becomes evident when that already in the blood is destroyed.

Icterus does not form part of the picture of hepatic insufficiency. It does not develop after partial removal of the organ, even when, following the technique of Mann and McGath, regeneration of the liver is prevented; it does not occur in any species of animal after hepatectomy, even when the period of survival is more than 20 hours as in the frog. When,

following intravenous injections of glucose a yellow pigment appears in the serum of the dog, which may, perhaps, be bilirubin, that only goes to prove, once more, that bile pigment may, in certain circumstances, be formed outside the liver; this does not show that the liver only eliminates a pigment elsewhere produced, and which accumulates in the body after removal of the gland.

If the above group of symptoms are rarely observed clinically, that is because a large part of the parenchyma of the liver can be removed without effect, and because regeneration of the gland takes place very rapidly, as Hanot and Kahn demonstrated long ago.

The organism is therefore well equipped against hepatic insufficiency. Thus, a destructive tissue lesion, such as occurs in Laennec's cirrhosis, can progress till death occurs, without at any time showing any real signs indicating hepatic insufficiency, and the fatal termination must be ascribed to toxic products liberated by the damaged liver(7).

The various symptoms described above always occur together, when they are caused by a more or less extensive resection of the gland. This is probably due to the synergy of the different functions of the liver, as physiologists have demonstrated.

Only the close study of these symptoms, combined with pathological examinations will tell whether there exist different forms of hepatic insufficiency (9).

CHROMAGOGUE FUNCTION TESTS.

The power of the liver to remove dyes from the blood-stream and then re-excrete them is merely one phase of the antitoxic function of the liver, and illustrates its ability to deal with foreign substances in the circulation. This function has been made the basis of the so-called dye tests.

In these tests, a given amount of dye is injected into a vein, and the rate of its removal from the blood noted, and also the amount excreted into the duodenum (via the bile), the intestines, and the urine.

The ideal substance for such a test should be non-toxic, a crystalloid, should remain in the blood stream sufficiently long and in sufficient concentration to be estimated, and the liver should be the only organ concerned in its elimination (10).

Such a substance must remain an ideal, at present, but the ones in use at present approximate more or less closely to the above requirements.

PHENOLTETRACHLORPHTHALEIN TEST.

This test originated from the observations of Rowntree & Abel (11) that when this substance was injected into the circulation, elimination took place solely by the liver. This at once suggested its use for the purpose of testing liver function.

On injection into the blood-stream, the dye at once commences to be taken up by the liver, but a small amount diffuses into the tissues, and is given up as the concentration in the blood-stream falls. It is excreted by the liver in the bile and, normally is not found in the urine, though in disease, varying amounts may be excreted by the kidneys.

In the original test, the amount recoverable from the faeces in the forty eight hours following injection was measured. This method was objectionable, cumbersome and inaccurate. The introduction of the duodenal tube technique brought about a modification of the test, and the amount recoverable from the duodenal contents was then used. This method is still followed

out by many, but an undoubted improvement was introduced by Rosenthal (12). This investigator measured the rate at which the dye leaves the blood-stream and this is the method employed at the present time.

Method:

The dye is made up in ampoules of 5% strength, each ampoule containing 10 mg. of dye. 5 mg. of dye are used for each Kg. weight and the requisite number of ampoules is dissolved in about 300 c.c. normal, sterile, saline. This solution is then injected into a vein, and the needle is then washed out with a few c.c. of saline to prevent local extravasation of the dye. Samples of blood are then taken fifteen minutes and one hour after injection, 5-10 c.c. being collected each time into a dry centrifuge tube, the blood allowed to clot, centrifuged, and the clear serum pipetted off.

It is very important to prevent haemolysis occurring, as this interferes with the estimation by colorimetry, and can only be avoided by allowing the clot to separate of its own accord. The percentage of dye present in each specimen of serum is then determined by matching against standards of known strength in a colorimeter.

For the purpose of the test, a series of standards must be obtained or prepared. It is best to prepare fresh standards every few days, as the colour deteriorates, and matching becomes difficult and inaccurate.

First of all, a 100% standard is prepared by dissolving the contents of one ampoule i.e. 0.2 c.c. of a 5% solution representing 10 mgm. of dye, in 100 c.c. distilled water. The colour developed after rendering this solution alkaline (by the addition of one drop of 5% NaOH) is taken as 100%, and represents the colour which would be given by the patient's serum, if no dye were excreted.

The following series of dilutions are made:-

Amount of 100% standard in c.c.	2.5	2.0	1.5	1.0	0.5	0.2
Amount of distilled water in c.c.	7.5	8.0	8.5	9.0	9.5	9.8
Resulting dilution of standard in percentage	25	20	15	10	5	2

One drop of 5% NaOH is added to each tube to render it alkaline. For matching the serum, some type of comparator should be used, that of Cole and Onslow being quite suitable.

The serum is divided between two test tubes of the type used in the comparator. To one is added one drop of 5% NaOH

but the other is left untouched unless haemolysis is present, in which case two or three drops of 5% hydrochloric acid are added. The alkaline serum is then matched in the comparator against the various dilutions of the standard prepared and the corres-:ponding dilution represents the percentage of dye remaining unexcreted in the serum.

Any colour due to the serum itself may be controlled by superimposing the second tube of serum on the standard in the comparator.

A simplification of the method for determining the amount of phenoltetrachlorphthalein has been devised by Bloom and Rosenau (13), which depends on the precipitation of proteins by acetone in the serum. An equal volume of acetone is added to the test-serum, and a heavy, white, flocculent precipitate results; this is centrifuged at low speed for two minutes when an almost crystal clear, slightly yellowish, supernatant liquid separates from the precipitate. This supernatant liquid does not give the biuret reaction. If phenoltetrachlorphthalein is present in the test-serum, it will remain in the supernatant liquid. The addition of three drops of 5% sodium hydroxide to this liquid causes the phenoltetrachlorphthalein to appear

with slightly more of a bluish tint than is present when the dye is in aqueous solution. A permanent standard for comparison with the test specimen can be made up in a manner similar to the method described above, except that the colour standard for each percentage dilution of the dye is diluted with an equal volume of acetone so as to compensate for the addition of acetone to the test specimen. The acetone standards begin to fade slightly after a month.

This method enables one to detect minute traces of the dye, and because of the sharp colour elicited from the sera by this technique, very accurate quantitative determinations can be made. Even the sera from heavily jaundiced patients give the same clear supernatant liquid; in these cases, the precipitate is coloured deep yellow, while the supernatant liquid does not give a colour reaction when treated with Ehrlich's diazo reagent (Van den Bergh's test).

In normal individuals from 2% to 6% of dye is found in the circulation after fifteen minutes, while after one hour none is present. In diseased conditions of the liver, the dye remains much longer in the blood, and may be recovered three hours after injection. A recovery of 8% or more after one hour is said to

be conclusive of impaired liver function (14).

Experimentally, it has been found (15) that 12% of liver must be removed to obtain a positive result.

The test is not free from danger, and local induration at the site of injection, venous thrombosis, rigors and chills may occur. Deaths have been reported a few days after injection (16, 17).

There can be no doubt as to the specificity of the test, except in obstruction of the biliary passages, when mechanical factors prevent the excretion of the dye, and falsify results.

The first examination, after fifteen minutes, can safely be omitted, and only the one hour sample taken and examined. This makes the test easier from the patient's point of view (18).

Marked retention of the dye in a case of enlarged liver would suggest a diagnosis of cancer rather than cirrhosis or hepatitis, as it has been found that the power of the liver to eliminate the dye is more affected in the case of the former disease (19). A positive result is therefore very valuable, but only gross liver damage can be indicated by this method, which fails to show very early lesions. A negative result does not exclude liver involvement, even of a fairly advanced type.

The large amount of dye which requires to be injected is another drawback (20).

The test is therefore not an ideal one, but very useful in the investigation of hepatic function.

BROMSULPHALEIN TEST.

This test was first introduced by Rosenthal & White, as an advance on the phenoltetrachlorphthalein test (21).

Method:

The dose is calculated on the basis of 2 mgm. of dye for each Kg. bodyweight. (Weight of the patient in lbs divided by 55 gives the number of c.c. of 5% solution required.) The dye is made up in ampoules of 5% strength. A 5 c.c. syringe should be used for the injection, which should take one minute, and the last drops of dye should be washed through the syringe by a few c.c. normal saline, to prevent infiltration of the dye outside the vein.

Thirty minutes after injection, 4-5 c.c. of blood are withdrawn preferably from the opposite arm, by allowing the blood to run through a needle into a dry test tube. In cases of early liver disease, it is recommended in addition that a sample be withdrawn five minutes after injection.

The blood is allowed to coagulate, care being taken to prevent haemolysis by not interfering with the process. The blood is then centrifuged, and the clear serum pipetted into two small test tubes. To one of these is added one or two drops of a 10% solution of sodium hydroxide, to bring out the colour of the dye, and to the other, a drop of 5% HCl to clear the serum of any haemolysis. The percentage of dye present in the serum is then estimated by comparison with a series of standards. The tube of clear acidified serum is placed in front of the standard in a comparator, and by simultaneously looking through both tubes, a comparison can be made with the coloured alkalised serum. The standards are prepared as follows: 4 mgm. of bromsulphalein are dissolved in 100 c.c. water rendered alkaline with 0.25 c.c. of 10% sodium hydroxide. This represents the 100% standard.

The following series of dilutions are made:-

Amount of 100% standard in c.c.	9	8	7	6	5	4	3	2	1
Amount of distilled water in c.c.	1	2	3	4	5	6	7	8	9
Resulting dilution of standard in percentage	90	80	70	60	50	40	30	20	10

5 c.c. of each standard may be sealed in a small test tube,

and no deterioration in colour will result for several months, if they are kept in the dark.

In normal individuals, an average of 35% is found in the serum five minutes after injection, the amount varying from 20% to 50%.

Thirty minutes after injection, the serum is entirely free from dye, or rarely, a very faint trace is present, the amount being insufficient to be estimated colorimetrically.

The amount of dye present in the urine is negligible, varying from none at all, to 0.5% of the quantity injected. The estimation in the urine may be carried out with the same standards used for the blood serum.

It is said that the percentage of dye present in the blood serum thirty minutes after injection, expresses directly the percentage of impaired liver function. Thus a finding of 50% thirty minutes after injection would indicate that the hepatic efficiency was 50% normal. Experimentally, if the liver be excluded from the circulation, a 100% finding is obtained.

In the case of liver disease, with abnormal retention of the dye in the blood, bromsulphalein is excreted in the urine in the twenty four hours following injection, in amounts varying

from a trace, to 20% of the amount injected. There is no correspondence between the degree of retention in the blood, and the amount excreted in the urine, so that, as with tetrachlorphthalein, its excretion in the urine cannot be taken as a quantitative test for liver function, although it may show striking variations from normal, in liver diease. Furthermore, in mild cases of impaired hepatic function, as shown by the bromsulphalein test, no abnormal excretion in the urine may be noted.

In no cases have untoward effects, such as have been observed with tetrachlorphthalein, been noted with bromsulphalein, so that the latter is in every way, a much safer dye to use. Further advantages are the smaller dosage of the drug, and the high concentration reached in the blood at the end of thirty minutes. This test is also a specific one, abnormal retention invariably implicating the liver. The test is comparatively free from technical error, the estimation being easy to make.

In comparison with phenoltetrachlorphthalein, it has been shown that the liver is more sensitive to bromsulphalein and positive results are obtained at an earlier stage than with the former compound.

The test is useless in the case of biliary obstruction, and it has been found that retention of the dye does not run parallel with the bilirubin retention (22). The test will not reveal very early hepatic dysfunction, so that the clinical signs of early hepatic disease may be present, and a negative result obtained. Some observers have obtained positive results, in a large number of cases with the laevulose tolerance tests, while with bromsulphalein the results were uniformly negative, (22) so that a negative result does not exonerate the liver.

The Bromsulphalein test, on the whole, is a valuable one, and in the opinion of some, is the best test of liver function (23).

ROSE BENGAL TEST.

Rose Bengal is, chemically, di-iodotetrachlorfluorescein of the triphenylmenthane series of dyes, and after injection into the circulation, is eliminated entirely by the liver, the urine remaining free from the dye except in rare cases (24). It thus resembles phenoltetrachlorphthalein very closely in its behaviour.

Another characteristic of the dye is its photodynamic activity, resulting in haemolysis on exposure to light. To reduce this factor to a minimum, the blood is collected and kept before centrifuging in a subdued light, and centrifuged as soon as possible after collection. As a precautionary measure the patient is kept in a darkened room for an hour following the injection of the dye, although it is improbable that sunlight would have a markedly deleterious effect on a patient into whom the dye had been injected.

The following method has several advantages: The patient is subjected to a single puncture of the vein; the dye is easy

to obtain in bulk, and can be sterilised without chemical change (25).

Method:

A vein in the cubital fossa is selected, and 10 c.c. of a 1% solution of rose bengal in physiological sodium chloride solution is injected, and the needle washed through slowly with from 5 to 10 c.c. of salt solution, which is held ready in a fresh syringe. The needle is left in the vein, and at exactly 2 minutes after injection of the dye, a sample of blood (10 c.c.) is withdrawn from the needle into a clean syringe and discharged into a centrifuge tube containing a few crystals of potassium oxalate. The tube is carefully inverted two or three times. The needle is again washed by slowly injecting from 5 to 10 c.c. of the salt solution, which manoeuvre prevents the clotting of blood in the needle. At eight and sixteen minutes, respectively, from the time of injection, samples of blood are withdrawn and collected in an identical manner. The needle is then withdrawn from the vein in the arm. The patient should be warned that the appearance of the dye in the stools will impart to them a distinct red colour, since the unexpected appearance of the red

stools may cause considerable alarm.

As soon as possible after collection, the blood samples are centrifuged at a speed of 2,000 revolutions per minute, for thirty minutes. 10 c.c. record syringes and 16 gauge record needles are convenient to carry out these operations. Rough handling of the blood samples is apt to cause some haemolysis of the red cells and to interfere with the colorimetric readings.

Colorimetric Analysis: 5 c.c. of the plasma of each of the samples is separately mixed with 10 c.c. of physiological sodium chloride solution. The diluted plasma of the two-minute sample is placed in the wedge of a Hellige colorimeter. This is used as the standard against which the eight and sixteen minute diluted samples are read.

Since the two-minute sample represents the highest concentration of the dye in any individual following the injection of a fixed amount of dye into the circulation, it may be regarded as 100% retention, and the colorimetric readings obtained by comparing the eight minute and sixteen minute specimens with it represent direct percentages of dye retained. Before making colorimetric readings, it is necessary to standardise the Hellige colorimeter used, and to correct for any inaccuracies present.

The colorimeter is standardised by comparing one sample of plasma against itself; the reading should be 100.

The colorimetric readings thus obtained indicate the ratio between the first or control sample and the subsequent samples. This obviously indicates the speed with which the dye leaves the circulation.

Although the two minute sample is taken as representing the highest concentration of the dye in an individual at any one time, it is probable that some of the dye is removed from the circulation before the collection of the two minute sample; but this is not of great practical importance if care is taken to withdraw the blood at exactly two minutes. Furthermore, we are not so much concerned with the actual quantity of dye in the circulation as with the ratio of the percentage of dye in circulation at two distinct intervals of time, which in reality is its rate of disappearance. For this reason it is not essential that the solution of dye injected be exactly a 1% solution nor that the dose be exactly 10 c.c. Although considerable latitude may be taken in regard to the amount of dye injected, it is advisable to inject approximately 100 mgm. in the average sized adult. The dose may be varied according

to the size of the patient, but some endeavour should be made to keep the initial concentration of the dye approximately the same.

The dosage may be varied from 3 c.c. of a 1% solution in children to 15 c.c. in large adults.

In normal individuals, the eight minute sample is usually 50%, varying from 40% to 60%, and the sixteen minute sample varying from 23% to 30%. These percentages represent the amount of dye retained in the circulation.

The sixteen minute specimen may be obtained by leaving the needle in the vein the entire time, or a fresh venipuncture can be made to obtain the sample (26).

Results:

In chronic cholecystitis the elimination is usually within normal limits, and the test is not an aid in the diagnosis of the condition.

In cases of obstructive jaundice, catarrhal jaundice, and arsphenamine icterus, the delay in the elimination of the dye is always very definite, being greatest in obstructive jaundice.

The test may be used as an aid in determining the patency

of a cholecyst-duodenostomy, as the dye can readily be indentified in the duodenal contents.

In cirrhosis of the liver, the impairment of liver function is in direct relation to the amount of scarring in the liver, and to the encroachment on functional reserve of the liver.

In cardiac failure, tuberculous peritonitis, and in carcinomatosis of the peritoneum, little change from normal is obtained.

In metastatic malignancy of the liver, the conditions are variable, and seem to depend on the amount of liver tissue which can function.

Chronic passive congestion of the liver does not seem to prevent the absorption of rose bengal, and the curves are within normal limits.

In eclampsia and toxaemias of pregnancy no retention of the dye is found.

In general, the rose bengal test for liver function gives results in direct proportion to the clinical and pathological data noted in regard to the liver. The technique of the test is simple and the concentration of the dye in the serum is great enough for the estimations to be easily performed.

Of all the dyes suggested, rose bengal seems to be the most satisfactory, and to yield the most consistent results. Retention of the dye is invariably associated with hepatic dysfunction, but a negative result does not exclude liver disease.

DETOXICATION TESTS.

The discovery of the detoxicating power of the liver for cyclic and other organic compounds had its origin in the researches of Wiedemann (27) who found that the administration of camphor led to the appearance in the urine of an acid substance, later identified by Schmeideberg and Myer (28) as campho-glycuronic acid. Following on this discovery by Schmeideberg, a whole series of compounds were discovered, which were capable of combining with glycuronic acid in the body after oral administration, among which may be mentioned chloral hydrate, orthonitrotoluene, phenol, phenethol, dichlorobenzene, xylol, cumol, butyl-chloral hydrate, terebene, menthol and thymol.

Glycuronic acid is an oxidation product of glucose, possessing the chemical structure of a glucoside, with a carboxyl group at one end of the carbon chain, and an aldehyde group at the other.

Since the subcutaneous injection of glycuronic acid leads to its elimination in the urine, this acid cannot be a

normal oxidation product of sugar.

In general, it may be said that in order that a substance should combine with glycuronic acid, it should contain a hydroxyl group. Those substances which do not contain a hydroxyl group must first be modified in the body by oxidation, reduction or hydration, so that a hydroxyl group enters the molecule. The combination between the two compounds then occurs between the hydroxyl group of the reacting substance, and the hydroxyl group of the aldehyde atom of the glycuronic acid, with the elimination of a molecule of water.

Alcohols and phenols can thus combine with glycuronic acid without preliminary modification. Camphor, on the other hand, must first be oxidised to camphrol before this can occur.

Glycuronic acid is only formed in the body from glucose when there is present in the organism any compound capable of uniting with it. Normally, human urine contains a small amount of glycuronic acid, combined with phenol, cresol and indol. Tollens and Stern, by their furfurol method of estimation found 0.025% present in normal urine (29).

The ingestion of salts of glycuronic acid leads to an

increase in the glycuronic compounds in the urine, without increasing the elimination of phenol and indol. On the other hand, this leads to a diminution of the sulphur excretion in the urine, and especially of the ethereal sulphates. There would thus appear to be a sort of equilibrium between the glycuronic and sulphur elimination in the urine.

In view of the fact that a whole series of toxic substances has been found to be capable of being eliminated combined with glycuronic acid, and also because of the relation between sulphur and glycuronic acid excretion, it would seem that a defensive mechanism is present in the body whereby toxic substances, of endogenous and exogenous origin, are eliminated in the form of glycuronic acid compounds. The traces which are normally present in the urine, and which owe their presence to the small quantities of phenol and indol formed in the intestines, are considerably increased in some affections, chiefly those affecting the digestive tract, such as constipation, ileus, and the presence of foci of suppuration. In other cases, the increase is not easily explained away by the increased formation of phenol and indol, such as in diabetes, pneumonia, and disorders of the circulation and respiration.

Regarding the site of formation of the synthesis of glycuronic compounds, it has not been definitely established that this is the liver, though the consensus of opinion is in favour of that organ playing the chief part, while other organs may have a subsidiary role. There is also some evidence suggesting that the mechanism may not be identical for all compounds of glycuronic acid (30).

THE CAMPHOR TEST.

As indicated above, when camphor is ingested, it is conjugated in the body, presumably in the liver, with glycuronic acid, which can be identified by means of the reaction described below.

Method:

The patient swallows, in the morning, fasting, 2 gelatine capsules each containing 0.5 gm. camphor in the form of camphorated oil, and the urine is collected in the following 24 hours. The urine passed immediately before the test is also collected, and both specimens tested for the presence of glycuronic acid.

To 5 c.c. of urine in a centrifuge tube is added 0.2 c.c. ammonia, and 2 c.c. of 10% basic lead acetate solution. A heavy precipitate is formed, which contains all the glycuronic acid. The tube is then filled with distilled water containing 1% ammonia, centrifuged, decanted, and washed twice with

ammonia water. To the precipitate are now added 5 c.c. distilled water, and the whole transferred to a test-tube. 0.5 c.c. of a 1% alcoholic solution of naphthoresorcin is added, and to collect the deposit which adheres to the sides of the centrifuge tube, 5.5 c.c. of pure hydrochloric acid are added to the latter, which is then emptied again into the test-tube. The test-tube is heated on a boiling water-bath for 15 minutes, then cooled in running water, 10 c.c. of ether are added, and well shaken-up.

If the urine contains no glycuronic acid, a light rose colour is obtained in the ethereal layer which is the colour obtained when naphthoresorcin is heated with hydrochloric acid.

If glycuronic acid is present, the ethereal layer takes on a violet tint, the depth of colour depending on the amount of acid present.

Certain precautions must be taken. The hydrochloric acid must be pure. The naphthoresorcin must be kept in the dark, or else it decomposes. Again, during the heating on the water-bath, the tube should not touch the sides of the latter, since a temperature of over 100°C will cause furfurol to be

formed by the action of the hydrochloric acid on the glycuronic acid, and a red colour thus imparted to the solution.

A quarter of an hour should elapse between the addition of the ether, and observing the result, as the reaction takes some time to develop. If difficulty is experienced in making the ether separate, the addition of a little salt water will expedite this.

Test-tubes of equal diameter should always be used, so that similar depths of liquid are compared.

The reaction can be made a quantitative one, by preparing the following standard solution corresponding to 100 mgm. of glycuronic acid per litre.

1% solution of neutral red	2 c.c.
Phenic alcoholic solution of gentian violet	1.5 c.c.
Distilled water	100 c.c.

A series of standards can be made from the above solution, and the ethereal layer compared with the naked eye.

Colorimetric estimation is more accurate. The ethereal layer is placed in one cup, and the standard in the other, the latter being set at a depth of 10 millimetres for strong

solutions, and at 30 millimetres for weak solutions.

If H is the depth of the unknown required to match the standard, and the glycuronic acid content of the unknown is represented by X, then we have:

$$\frac{X}{100} = \frac{10 \text{ or } 30}{H}$$

Results:

Normally, 0.01 gm. to 0.04 gm. glycuronic acid per litre are found. During a fast, or on a milk diet, the result is negative, and rises on a meat diet.

Normally, after administration of camphor, the concentration rises to 0.08 gm. per litre in the first four hours, falling to normal 6 - 8 hours later.

Conclusions:

There is a great difference of opinion as to the practical value of the camphor test. There can be no doubt that it is but a crude test of hepatic function, while, in addition, our absence of knowledge concerning its precise mechanism detracts from its significance.

Rogers (31) in a large series of cases, has found that a diminished glycuronuria is always associated with hepatic deficiency, and his conclusions are confirmed by Gautier (32) who ascribes a prognostic value to the test in finding that in cirrhosis of the liver, the total absence of glycuronuria is a sign of a speedily fatal outcome. The test is also advocated by Dodds (33) and by Van Dooren and Destree (34).

On the other hand, Schmid in a series of cases, found the test of no value. He says: "Nous pensons que l'épreuve de la glycuronurie provoquée n'est pas susceptible de nous renseigner sur la fonction du foie".

The specificity of the test is undoubted in advanced liver disease, but in early and doubtful cases, the results are equivocal, since a small amount of liver tissue is apparently able to ensure the conjugation with glycuronic acid.

THE MENTHOL TEST.

The administration of menthol is unattended by the objectionable subjective sensations accompanying the ingestion of camphor, while symptoms of poisoning are notoriously absent. The menthol combines with glycuronic acid in the body without preliminary oxidation, and is excreted in the urine in this form. Thus two of the chief objections against the use of camphor are not present in the case of menthol.

Method.

The menthol is administered in the form of capsules containing the pure crystalline compound, 2 g. being swallowed several hours after breakfast, and the urine collected during the following 24 hours (35). The urine is examined for the presence of glycuronic acid by the Roger's reaction, and quantitative estimation can be carried out by the methods described in the case of camphor.

Results:

Schmid, who examined a series of cases, found no instance in which glycuronic acid failed to appear in the urine after the administration of menthol. While, normally, the excretion of glycuronic acid is complete within 24 hours after the test, in some cases, there was delay, and the elimination continued over several days, e.g. 2 days in generalised tuberculosis, 4 days in icterus gravis, 2 days in catarrhal jaundice during the icteric period, and 2 days in syphilitic icterus. In a fatal case of icterus gravis, a greatly increased glycuronuria occurred. This would seem to support the views of Pick and Pohl, that in the presence of severe lesions of the liver, the formation of glycuronic acid takes place in other organs.

The conclusions, quoted above by Schmid with reference to camphor, apply also to menthol. No information of diagnostic or prognostic significance can be obtained by the use of the menthol test.

THE SALICYLATE TEST.

When sodium salicylate is introduced into the body, it is excreted in the urine combined with glycuronic acid. If, however, the liver is deficient, part of the salicylate is excreted unchanged and can be identified by chemical methods. The basis of the test is, therefore, the same as the camphor and menthol tests, only, instead of examining for and estimating the glycuronic acid, we test for the elimination of the original compound administered.

Method:

One hour after breakfast, say at 8 o'clock, 4 c.g. (about 0.5 gr.) of sodium salicylate is swallowed and the urine passed between 9 and 11 o'clock, and between 11 and 1 o'clock, collected in separate specimens. The urine is added, drop by drop, to a test tube containing a 1% solution of perchloride of iron. The formation of a violet colour at the junction of the two liquids is a positive reaction,

and indicates the presence of salicylate.

Results:

The normal liver is capable of retaining or transforming 4 c.g. of sodium salicylate, or at least, of not allowing it to pass in such quantities as will give the reaction with perchloride of iron.

On the other hand, if the liver is diseased or deficient in its function, enough salicylate is excreted as will give a positive reaction.

Roch says of the test (36) "Elle est d'une grande simplicité, et d'une parfaite innocuité, et dans une pratique déjà étendue, elle nous a fourni des resultats très encourageants".

Further work should be done on this test which has given such encouraging results, and of which the technique is so simple and conclusive. It is doubtful, however, whether it can indicate the presence of early hepatic lesions.

THE THYMOL TEST.

Besides the detoxicating power of the liver by means of glycuronic acid described above, another mechanism has long been known. This is the union of aromatic radicals with sulphuric acid, and their excretion in the form of conjugated sulphates (ethereal sulphates) in the urine.

In the colon, during the course of digestion of proteins, carbocyclic toxic compounds, such as phenol, cresol, indol, and skatol, are formed by the action of the intestinal bacterial flora acting anaerobically on proteins. These products are very poisonous, but when they are united with sulphuric acid they lose their poisonous effect.

There is no consensus of opinion as to which organ is primarily concerned in this chemical process, but most observers are of the opinion, although the experimental evidence is inconclusive, that the liver is chiefly responsible for the mechanism by which these toxic substances are rendered innocuous.

Normally, the inorganic sulphates of the urine form about

70% of the total sulphur excreted, while the remaining 30% is equally divided between the ethereal sulphates and the neutral sulphur. It is, of course impossible to rely upon the excretion of ethereal sulphates as an index of hepatic function, since the proteins, which are ingested daily, give rise to their quota of aromatic radicals which influence the quantity of the conjugated sulphates excreted.

Nevertheless, in hepatic disease, there have been observed disturbances in the elimination of ethereal sulphates, for example, icteric patients usually show an increased excretion of skatol and indol, while the ethereal sulphates are, both relatively and absolutely, increased in hepatic cirrhosis and tumours of the liver. It is therefore probable, that, under normal conditions of hepatic activity, the aromatic compounds formed in the system are diminished in amount and destroyed, while in cases of disturbances of the functions of the liver these compounds are increased, and placed at the disposal of the liver for conjugation with sulphuric acid. It has been found that the administration of thymol leads to an increase in the ethereal sulphate content of the urine. Thymol is meta-isopropyl-cresol, and is eliminated as

thymol-sulphuric acid and as thymol-hydroquinone sulphuric acid.

The administration of thymol is really a test of the power of the liver to conjugate thymol with sulphuric acid and thus increase the ethereal sulphates in the urine.

Method:

The patient receives a dose of castor oil to clear out the bowels. He is then kept on a known diet for 2 days during which time the urine is collected, and preserved with a few drops of formaldehyde. The urine is then analysed for its total sulphur content by Benedict's method and the ethereal sulphates estimated by Folin's method. On the third day, the patient receives a capsule containing 0.5 g. thymol. A dose of olive oil is administered to the patient several hours after the thymol dose in order to dissolve the thymol, and increase its absorption from the alimentary canal. The urine is collected for the next forty-eight hours, preserved with a little formaldehyde, and analysed for total sulphur and ethereal sulphates.

If all the thymol were absorbed and if all the thymol

were conjugated with sulphuric acid, and none with glycuronic acid, the 0.5 g. thymol would be excreted as 0.7666 g. thymol-sulphuric acid, and would cause a marked increase in the percentage of ethereal sulphates.

Results:

Kahn in a series of cases (37) obtained positive results in cases of cancer of the liver, atrophic cirrhosis and syphilis of the liver yielding the most marked results. Thus, while, in normal cases, the ethereal sulphate percentage was more than doubled, the above cases showed only a slight increase after ingestion of thymol.

The test contains several inherent fallacies and sources of error. In the first place, hepatic conditions usually lead to such digestive disturbances as upset the normal putrefactive processes in the intestines, notably when the secretion of bile is defective, and thus lead to an upset in the normal sulphate excretion. This is guarded against, in the test, by placing the patient on a known diet, and analysing the urine before performing the test.

Again, the detoxicating power of the liver manifests

itself not only by sulphuric acid conjugation, but also by glycuronic acid conjugation, and, so far as we know, there may be other mechanisms at work of which at present we have no knowledge. In the above test, as performed by Kahn, no note is taken of the glycuronic acid excretion, and it is probable that simultaneous estimation of glycuronic acid and ethereal sulphate excretion would make for increased accuracy. This would make the test rather complicated.

Sufficient work has not been done to justify any definite conclusions as to the value of this test, but it does not appear to hold out much hopes of its proving of any value.

PIGMENTARY FUNCTION TESTS.

Bilirubin is the most important of the bile pigments, and gives the bile its characteristic colour. It is a crystalline compound, reddish yellow in colour, insoluble in water, soluble in chloroform, alcohol, and alkalies. It is a weak, dibasic acid, and forms salts. It occurs in bile as the alkaline salt. Chemically, it may be classed as a chromoprotein.

The origin of bilirubin from the haemoglobin of the blood has been demonstrated physiologically and chemically. In localised haemorrhagic effusions haematoidine is formed, which is a pigment closely related to bilirubin. During haemolysis, whether occurring in pathological processes or experimentally induced, the quantity of bilirubin in the blood is increased in proportion to the degree of haemolysis.

Treating haematin and bilirubin with oxidising and reducing substances gives rise to closely-related chemical compounds. Thus, by reduction of haematin, various bodies

containing the pyrrol nucleus are obtained. The oxidation of haematin or bilirubin gives rise to a tribasic haematinic acid. The chief difference between haematin and bilirubin is that the former contains iron, which is absent in the latter. Schematically, the following equation represents the relation between these two bodies:

$$\text{Haematin} + 2H_2O = \text{Bilirubin} + Fe$$

It was formerly thought that the sole seat of origin of the bile-pigments was the hepatic secreting cell. Recent work has thrown doubt on this conception, and tended to show that the bile pigments may also have an extra-hepatic origin. The following are the chief arguments in favour of the modern view:

1. The blood contains an amount of bilirubin which is kept relatively constant by the continuous hepatic excretion of bilirubin. In biliary obstruction bilirubin accummulates in the blood, and when it exceeds a certain limit, is excreted by the kidneys. Thus bilirubin behaves like a substance having a threshold of excretion. These facts are difficult to reconcile with the theory of the hepatic origin of the bile pigments, and it would be necessary to postulate that the hepatic cell eliminated its pigment by two distinct routes, biliary and vascular.

2. Certain hepatic poisons can cause jaundice. It would

appear from this that the damaged hepatic cell produces an abnormal amount of bile-pigments, unless one were to invoke the conception of a break in the continuity of the bile-capillaries, allowing the bile to enter the circulation directly.

3. In certain animals, jaundice has been produced after complete hepatectomy. The jaundice is, however, admittedly very slight, and the experiments have not been confirmed.

After the bile reaches the intestines, it is in part excreted in the form of stercobilin, and in part re-absorbed, and, reaching the liver, is again excreted, this process forming an entero-hepatic circulation. The exact function of the pigments is but little understood. They behave like waste-products which the body is constantly excreting. They are, in fact, toxic, from five to ten times more toxic than the biliary salts. They lower the temperature, and can give rise to various nervous manifestations.

THE VAN DEN BERGH REACTION.

The modern conception of the origin of the bile pigments originated with the work of Van den Bergh (38), and it is now assumed, on experimental evidence, that it is the reticulo-endothelial system of cells which convert the blood pigments into bile pigments. This system of cells is widely spread throughout the body and the cells are particularly abundant in the spleen, which indicates the possibility of specialised functional activity of parts of the system. The kupffer cells of the liver are also a part of the reticulo-endothelial system. The haemoglobin in the blood stream is thus converted into bile in the kupffer cells, which normally pass it on through the polygonal cells, to the lumen of the bile capillaries. The functions of the polygonal cells, therefore, are simply transmission, and they are not essentially concerned with the production of pigments.

It is thus possible to have jaundice arising in one of three ways:

1. Where obstruction of the bile tract prevents the escape through the natural channels. The bilirubin so formed will then be reabsorbed into the blood-stream, to be subsequently excreted in the urine.

2. Where the polygonal cells lose their power of transmitting pigment to the capillaries, with the result that it passes direct from the kupffer cells to the blood-stream. A similar series of events might arise if, through excessive blood-destruction, more pigment were offered than the polygonal cells could pass through. Some would then be absorbed direct into the blood-stream, and the rest would pass normally into the biliary passages.

3. Where there is a combination of these two conditions. That is to say, although the polygonal cells are damaged, some bilirubin might pass through into the biliary passages, from which, owing to obstruction, it would be absorbed into the blood-stream. In addition to this, there would be direct absorption from the kupffer cells.

Thus, in purely obstructive jaundice the bilirubin has passed through the polygonal cells of the liver, but it is possible to have icterus due to bilirubin which has not passed

through these cells.

Accordingly, the following classification of jaundice is now generally accepted (39).

1. Obstructive Hepatic Jaundice:- This variety arises in the liver by obstruction either temporary or permanent, gradual or sudden, of the bile passages, for example in cases of impacted calculus, or neoplasm in the common bile duct.

2. Toxic and Infective Hepatic Jaundice:-

This includes the jaundice which occurs:

(a) As a complication of many acute fevers - pneumonia, typhoid, spirochaetal jaundice.

(b) After the administration of certain drugs - chloroform, salvarsan, phosphorus.

(c) After damage to the liver cells of an unknown kind - acute and subacute atrophy of the liver.

(d) After many general conditions of infection and toxaemia - acute sepsis, cardiac valvular disease with "back-pressure".

3. Haemolytic Jaundice:- This includes the group of "liver-spleen diseases", - pernicious anaemia, splenic anaemia, Banti's disease, in some of which the icterus is definite, in others, of the latent type.

The Van den Bergh reaction was originally introduced by Van den Bergh as a means of differentiating the various types of jaundice, and was later extended to give a quantitative estimation of bilirubin in the blood. The reaction depends on the fact, first noted by Ehrlich that bilirubin when dissolved in chloroform or alcohol, gives with diazonium salts a reddish colour in neutral solution, and a bluish colour in acid solution. Van den Bergh found that no other substance likely to be present in the blood, will give the reaction, and he has never detected any substance in human serum, except bilirubin, which gave the reaction. Bilverdin does not react to the test. In addition, lutein which in certain diseases may deeply colour human blood serum, and even give the appearance of jaundice to the skin, does not give a positive result.

Method:

The examination must be made within about two hours of taking the blood, or anomalous results may be obtained. Thus sera which gave a prompt direct reaction, when tested immediately after withdrawal, gave a slow and long-drawn out result when retested twenty four hours later (40). Differences

in reaction to the acid or alkaline side are also of great importance in the development of the colour of the azo-bilirubin compound. Haemolysis also interferes with the colour-reaction of the test, so that oxalated plasma should always be used.

About 10 c.c. of blood are collected in an oxalated tube. The latter is prepared by adding 0.2 c.c. of 10% potassium oxalate solution, and evaporating to dryness during the process of sterilisation. The oxalate is in this way finely distributed over a large surface, and will prevent coagulation if shaken. The tube containing the blood is centrifuged, and the clear supernatant fluid is pipetted off.

The following solutions are required:-

(1) A freshly prepared solution of Ehrlich's diazo reagent. This is made by mixing the two following solutions immediately before the test:-

A.	Sulphanilic Acid	1 g.
	Concentrated HCl	15 c.c.
	Distilled Water.	1,000 c.c.
B.	Sodium Nitrite	0.5 g.
	Distilled Water	100 c.c.

The actual reagent is made immediately prior to the test by

mixing in the proportions of 25 c.c. of solution A, to 0.75 c.c. of solution B.

(2) Absolute or 96% alcohol.

1. The Direct Reaction:

Three small tubes are set up, numbered 1, 2, and 3, and 0.25 c.c. of oxalated plasma is pipetted into each. To the first tube, the control, 0.2 c.c of water is added while into No. 2, a small flake of caffeine-sodium salicylate is dropped, which is said to accelerate the development of the colour-change. After the salt has been dissolved by shaking, 0.2 c.c. of fresh diazo reagent is added, and any resultant colour change allowed to reach its maximum. 0.2 c.c. of the diazo reagent is then added to tube No. 2, and any alteration in colour is compared with the fully developed reaction in tube No. 3.

One of three events may occur in tube 2.

(a) An immediate direct reaction:-

This begins at once, and reaches a maximum in 10 to 30 seconds. The mixture turns to a bluish-violet colour, the intensity varying as the concentration of bilirubin.

(b) A delayed direct reaction:-

This begins after 1 to 15 minutes, or even longer, and consists in the development of a reddish coloration, which gradually deepens, and becomes more violet.

(c) A biphasic reaction:-

In this, a slight reddish colour appears immediately (ten to thirty seconds), which after a minute, or much longer time, is seen to deepen gradually, and become more violet.

According as the reaction approaches more closely to the prompt or delayed types, a division is made into biphasic prompt and biphasic delayed reactions.

The above time limits for the reactions to appear are quite arbitary and different observers obtain varying reaction-times, depending on the reagents used. Ultimately, the interpretation becomes a matter of individual experience.

(2) <u>The Indirect Reaction</u>.

If an immediate direct or delayed direct reaction is not obtained, the indirect reaction should be proceeded with.

To 1 c.c. of plasma, 0.5 c.c. of diazo reagent is added, and after a minute or so, 2.5 c.c. of 96% alcohol and 1 c.c. saturated aqueous ammonium sulphate solution are added.

After thorough mixing, the tube and contents are centrifuged, and the supernatant clear fluid can be used for quantitative colorimetric analysis. If the colour be too deep, it may be diluted with alcohol, two parts to one of the solution.

A layer of clear ammonium sulphate solution is left at the bottom of the centrifuge tube, above this, a white albuminous precipitate, and on the surface, the clear, reddish-violet alcoholic solution of azo-bilirubin.

Quantitative Analysis:

The indirect reaction may be converted into a quantitative process by matching against a standard of ferric thiocyanate in ether. This solution is made up as follows:-

Dissolve 0.150 g. of ammonium iron alum in 50 c.c. of concentrated HCl, and add water to 100 c.c. The resulting solution is quite stable. To 10 c.c. of this solution add 25 c.c. of concentrated HCl, and water up to 250 c.c. This gives a dilution of 1 in 8,000 N, which keeps for about six months. To 3 c.c. of this stock solution, an equal volume of 20% potassium thiocyanate solution is added, and the resultant mixture is well shaken, and extracted with 12 c.c. of ether.

The ethereal extract is used as the standard, and the solution obtained in the indirect test is matched against it. This standard corresponds to a concentration of 1 in 32,000 N of bilirubin, i.e. 1 in 200,000 azo-bilirubin. An indirect test, containing exactly the same amount of colour as the standard, is said to contain 1 unit of bilirubin.

A new artificial solution has been suggested by Van den Bergh & Muller, on account of various difficulties encountered in using the ethereal standard solution. The new standard is made by dissolving 2.161 g. of anhydrous cobaltous sulphate in 100 c.c. of water thus giving a solution which corresponds in colour with azo-bilirubin solution of 1 in 200,000, i.e. one unit. The salt must be anhydrous, and the standard solution should be checked against the one already described, and stored in the dark. In this way, it is available at any time, without deterioration taking place.

Any simple form of colorimeter may be used, but even with the best technique, the results are of an accuracy which is adequate for clinical purposes only. There are various fallacies which prevent a completely accurate estimation of the whole of the bilirubin present in any serum. One of the chief of these

depends on the fact that some bile pigment is always carried down in the albuminous precipitate when alcohol is added. The amount, however, is always small, and is greater in cases of obstructive than of non-obstructive jaundice.

An important point in the colorimetric test is to take note of the amount of dilution of the serum necessary in the various steps of the test. The dilution of the original serum is one fifth, and this must be taken into consideration in making the final calculation.

Results of the Test.

This test has established the fact that normal human blood-serum always contains a small amount of bilirubin, which is only detected by the indirect reaction. Taking as the unit of bilirubin the figure 1 in 200,000, the normal physiological limit for the bilirubin content of human blood-serum is found to be between 0.2 and 0.5 unit.

The threshold value of the kidney for bilirubin in obstructive hepatic conditions has been found to be 4 units. Below this figure biliuria is absent, and a condition of latent obstructive jaundice exists.

The immediate direct reaction is given only by bilirubin which has passed through the polygonal cells of the liver into the bile capillaries, and has then been reabsorbed into the blood. Hence, an immediate direct reaction indicates the presence of obstructive jaundice, whether this be caused by a stone in the common bile-duct, by tumours, enlarged glands in the portal fissure, hepatic cirrhosis, etc.

The delayed direct, or the indirect reaction indicates a haemolytic, or non-obstructive cause for the jaundice. It must be noted, however, that all sera giving a positive direct reaction, will also give an indirect reaction, though the reverse is not necessarily true. Thus, pernicious anaemia, except in the stage of rapid temporary improvement, gives the delayed direct or indirect reaction, and can thus be distinguished from the secondary anaemias, and also from a frank obstructive jaundice.

The biphasic reaction is given where there is a combination of obstructive and functional causes at work, and includes the large category of toxic and infective hepatic jaundice. As this is the commonest form of jaundice occurring clinically, very little information of diagnostic or prognostic value is

obtained by this reaction (40).

In some diseases, for example, catarrhal jaundice, subacute liver atrophy, cardiac failure with hepatic enlargement, the reaction may pass through all the stages, from completely delayed to prompt direct, as the disease progresses. This is another great weakness of the test, from the diagnostic viewpoint.

Some of the advantages of the test may be summarised as follows:

1. It is the most delicate test known for the detection of bilirubin in the blood.
2. It is not given by any other yellow substances which may colour the plasma.
3. It gives, within certain limits, important qualitative distinctions between certain forms of jaundice.
4. It can be used for a quantitative estimation of bilirubin.
5. It is of great practical value in the detection of latent jaundice.

By latent jaundice is understood a retention of bile pigment (and frequently of bile acids as well) in the blood-serum, insufficient in amount to colour the skin and mucous membranes, and not giving rise to biliuria. The absence of bile in the urine pre-supposes, in the case of latent obstructive

jaundice, an amount of bilirubin in the blood of less than 4 units. In the case of latent haemolytic jaundice, this does not hold, as bilirubin is not excreted by the kidney in haemolytic conditions.

It must be admitted that the hopes originally entertained for the value of the test have not been substantiated, and as a diagnostic measure, it has very limited application. Thus in a particular case, if there is a question of diagnosis between catarrhal jaundice and stone as a cause for the icterus, a well-marked indirect reaction would indicate catarrhal jaundice rather than stone, while the presence of a direct reaction might mean either.

From the point of view of prognosis, no value attaches to the test.

THE BILIRUBINAEMIA TEST.

1. Van den Bergh's Indirect Reaction.

This method is described under the technique of Van den Bergh Test.

2. Icterus Index.

In this method, it is assumed that the yellow colour of the serum is due to bilirubin alone. The method was originally introduced by Meulengracht (41) and extended by Mave (42) and Bernheim (43,44).

Method:

The standard consists of a 1 in 10,000 solution of potassium bichromate, which is placed in one cup of the colorimeter, and set at 15 mm. depth. Into the other cup is placed the serum. If the depth of the unknown required to match the standard is x mm., the Icterus Index is $\frac{15}{x}$.

Normal serum gives an icterus index varying from 4 to 6.

3. Biliary Index of the Plasma (46).

In this method the colour of the plasma is compared with a solution of potassium bichromate.

Method:

The blood is withdrawn into a graduated centrifuge tube containing 2 c.c. of potassium oxalate solution, and centrifuged till clear. The levels of the corpuscles and plasma are read off, and the plasma is then compared colorimetrically with the following standard.

1 in 10,000 solution of potassium bichromate 100 c.c.
1 in 10,000 solution of orange-pear 2 c.c.

A correction has to be made for the oxalate. The total volume in the tube, V.t., and the volume of corpuscles, V.g. being known, the volume of oxalated plasma is V.t. - V.g., and the volume of the plasma is V.t. - V.g. - 2 c.c. The colorimetric reading must be multiplied by the fraction - $\frac{\text{Oxalated plasma}}{\text{plasma}}$ or $\frac{\text{V.t. - V.g.}}{\text{V.t. - V.g. - 2}}$

Normally, the Biliary Index of the Plasma varies between 1.6 and 1.8.

4. Rouillard (47) and Wauthier (48) compare the oxalated

plasma with a 1 in 5,000 solution of potassium bichromate. The solutions are placed in two Gowers haemoglobinometer tubes, and the plasma diluted till a match is obtained.

Results of the Bilirubinaemia Test.

The amount of bilirubin in the blood depends on various factors, haemic, hepatic and biliary. Clinically, it is exceptional for only one of these factors to be at fault. Thus in typhoid fever or in pneumonia the toxins elaborated lead to hyperbilirubinaemia by causing degeneration of the hepatic cells, and also, though in lesser degree, by haemolysis. Conversely, it is difficult to conceive of a toxic or haemolytic icterus without participation of the liver. Similarly, obstructive jaundice, at first acting entirely mechanically, later leads to hepatic degeneration, thus secondarily increasing the icterus.

Hypobilirubinaemia is found in secondary anaemias, and after a very severe haemorrhage. It is most marked in repeated, though small, haemorrhages such as occur in gastric and duodenal ulcer. It also occurs in malignant disease through the toxic action on the blood elements, provided the

liver is not the organ affected.

In chlorosis and in mild aplastic anaemias, the bilirubin is decreased. In kidney affections it is also slightly below normal, partly on account of the hydraemia, and partly due to increased resistance to disintegration of the red cells which is present (49).

In the case of the primary anaemias, the bilirubin in the blood is more or less increased, up to 3 units (50), thus enabling a differential diagnosis to be made at once between the secondary anaemias. The test is also of value in prognosis, as the cholaemia diminishes during the intermissions, the fall being proportional to the degree of improvement (51).

In cirrhosis of the liver, even in those unaccompanied by jaundice, the blood bilirubin is always increased (52,53,54). The test is also of value in prognosis of these affections.

In mild degrees of hepatitis such as occur in alcoholic polyneuritis, the test is positive, and again has a prognostic value.

Cancer of the liver and hydatid cyst, in the early stages, do not cause an increased cholaemia. However, the test is of value in the differential diagnosis of growths in the

hepatic region, such as right-sided hypernephroma, since, in the case of extra-hepatic malignant tumours, a hypobilirubinaemia is invariably present.

In the diagnosis of large irregular livers, in the absence of jaundice, the bilirubin is much higher in the case of cirrhosis than in secondary growths (55).

In catarrhal jaundice, the blood bilirubin is raised, but not usually very greatly, since the obstruction is often incomplete. In jaundice due to compression by a growth the bilirubin in the blood increases rapidly and regularly. In obstruction due to calculus, the bilirubin is raised but varies from time to time.

In infective conditions, it is increased, doubtless due to hepatic involvement. It is of special value in the estimation of latent jaundice occurring in these conditions. Thus in pneumonia, the advent of jaundice is always of grave import. In fatal cases of pneumonia, those without jaundice always had an icterus index over 7.5 (44); similarly in streptococcal pneumonia, a raised icterus index has the same prognostic value (56).

In typhoid fever, the presence of raised blood bilirubin

is also a bad prognostic omen. It is also increased in malaria (57).

In cholecystitis, increased cholaemia is present, while in cholelithiasis, it is always raised after an attack of colic, and sometimes even during the latent periods (58). It is thus of value in the diagnosis of right-sided abdominal pain, since a high icterus index would exclude renal or appendicular disease. However, in appendicitis with complications, a raised cholaemia is always present, but in salpingitis, the latter is always normal.

Duodenal ulcer gives a raised icterus index (44), while in gastric ulcer, it is normal. Low values, however, are found in the case of repeated haemorrhages.

In cardiac affections the blood-bilirubin rises when decompensation occurs, and if the cholaemia increases steadily, the condition is usually fatal.

In diabetes, cholaemia is usually increased, indicating hepatic involvement. In pregnancy, it is also raised, especially during the later months (59).

In syphilis, the blood bilirubin is normal, unless in the case of arsenical reactions, when it is always raised (60).

The bilirubinaemia test is thus of great value in the diagnosis of certain diseases of the blood, liver and biliary tract. It has a prognostic value if constantly repeated in the course of these affections. It is simple to perform, the technique requiring little skill. The test should be a routine one in the investigation of hepatic affections.

THE CHOLAEMIA TEST.

The presence of bile-salts in the urine has the effect of lowering the surface tension, as may be determined by stalagmometry. In an analysis of 400 urines by this method, Gilbert, Chabrol and Bernard found:

1. When the surface tension was below 850, 90% of the patients were suffering from an affection of the liver or biliary passages.
2. Between 850 and 900, 60% of cases had disease of the liver.
3. Between 900 and 1,000, the liver was the cause in 30% of cases.

Thus, any urine having a surface tension of 850 or under very probably contains bile salts. This figure is of importance, since the cholaemia test is only applicable to cases where there is no marked lowering of the surface tension of the urine, and where no hyperbilirubinaemia is present.

Method:

The amount of bile salts (glyco- and tauro-cholates of soda)

injected intravenously is 4 c.g. per kilo. of body-weight; usually, 2 - 3 gm. of bile salts dissolved in 10 c.c. physiological serum are injected. This quantity has no effect on the normal individual, but in persons with hepatic disease, it is not uncommon to have a rise in temperature, preceded by rigors, and accompanied by sweating, in the two hours following the injection; this, however, does not last long, and is not followed by sequelae.

The twenty-four hours urine before and after injection is measured for its surface-tension. Normally, the surface tension returns to normal within four to eight hours after the injection.

In pathological conditions, the elimination of the bile-salts is very slow, although all trace of them has disappeared from the blood-serum one hour after injection. By measuring the surface-tension of the urine, bile-salts can be shown to be present in some cases as long as 72 or 96 hours after injection.

Just before the test, a sample of blood is withdrawn, and the bilirubin measured by the Van den Bergh method. Other samples of blood are taken 4 and 6 hours after the

injection, and the bilirubin again estimated.

Normally, no difference is observed in the different specimens of blood withdrawn.

In hepatic affections the injection of bile salts causes an excess of bile pigments to appear in the blood; this is easily visible to the naked eye in the colour of the blood-serum, which becomes a lemon yellow or golden-yellow colour. This hyperbilirubinaemia may be due to the toxic action of bile salts on the hepatic cells, causing a mild and transient hepatitis, or else it may be caused by haemolysis (61).

Conclusion:

This test is still in the experimental stage, and further work requires to be done before its value can be estimated. The test does not appear to be without risk (62).

INDUCED BILIRUBINAEMIA TEST.

(Bergmann-Eilbott)

This test depends on the fact that a small amount of bilirubin introduced into the circulation is excreted by the liver only. The time of elimination depends on the functional condition of the liver parenchyma; the greater the damage to the latter, the more slowly is the bilirubin eliminated (63). The test is of no value if bilirubinuria is present.

Method:

0.07 gm. of bilirubin are dissolved in 10 c.c. of a 5% soda solution heated to 80°C to aid solution of the bilirubin. It is unnecessary to sterilise this solution, owing to the method of extraction of the pigment (by boiling ether). The solution is injected into a vein as soon as possible after preparation, preferably in the morning, fasting. A specimen of blood is withdrawn immediately before the injection; a second specimen is taken three minutes after the injection, and a third, 4 hours later.

The estimation is carried out according to the technique of Ernst and Foster. To 4 c.c. of blood is added 1 c.c. of a 3.6% solution of sodium citrate, to avoid coagulation, and centrifuged. Acetone is added to the plasma thus obtained, in the proportion of one part acetone to two parts plasma. A precipitate is formed, which is again centrifuged. The supernatant fluid, which contains the bilirubin, is compared with the standard, a 1 in 6,000 solution of potassium bichromate, in a colorimeter, as soon as possible.

The injection of the solution of bilirubin should be performed very shortly after preparation of the solution, in which case no reactions occur. If the injection is delayed for some time, rigors and slight elevation of temperature occur, which, however, are only transient, and never last longer than one hour.

Results:

The increase in bilirubinaemia 3 minutes after injection is taken as representing 100%. Thus, if the fasting bilirubin was 0.5 unit, and 3 minutes after injection 2.5 units, then the difference, i.e. 2 units, is taken as the 100% standard, since this

represents dilution of the injected bilirubin in the circulating blood. If a subsequent reading gives 1.5 units, the increase is 1 unit, and represents 50%.

In the normal person, the bilirubin in the blood falls to its former level in about 4 hours, but a reading of under 10% is taken as coming within normal limits (64,65). Any reading over 10%, 4 hours after injection, is pathological (66).

The test is strictly hepatic, and does not involve participation of the reticulo-endothelial system, as other dye tests may do. The test has given positive results in all diseases involving the parenchyma of the liver, and has demonstrated the existence of a hepatitis in cholecystitis, which other tests have hitherto failed to do. (67).

Conclusion:

The test is still in the experimental stage. It has the great advantage of being strictly physiological, and of involving only the liver cells. It would seem to be an ideal method for testing the power of the liver to excrete bilirubin. Further work may establish it as a routine method for testing liver function (68,69).

PROTEIN METABOLISM FUNCTION TESTS.

Physiological Considerations:

The proteins ingested in the food are ultimately transformed in the digestive tract into amino-acids. But the disintegration of proteins may, in certain cases, be arrested at the stage of polypeptides, without reaching the stage of amino-acids. The liver arrests the further passage of these polypeptides, which are more or less toxic, and decomposes them into amino-acids. Those amino-acids which are not required by the body for tissue-synthesis, as well as those of endogenous origin formed by tissue-disintegration, are destroyed by the liver. The nitrogen liberated in the course of this amino-acidolysis is employed by the liver for the synthesis of urea. This ultimate and least toxic form of protein decomposition then enters the general circulation, to be eliminated. The two chief functions of the liver with regard to proteins are thus, destruction of amino-acids, and formation of urea.

The first stage in the decomposition of amino-acids is deamination. This takes place by oxidation of the amino-acid, with production of ammonia and the corresponding ketonic acid, according to the formula:

$$R.CH.NH_2.COOH + O = R.CO.COOH + NH_3.$$

The fatty ketonic acids are broken down chiefly in the liver, by progressive oxidation, into carbon dioxide and water, but the intermediate steps vary with the different amino-acids. Some are decomposed like the sugars, while others are broken down like fats into aceto-acetic acid; these latter are thus ketogens. Others, again, are decomposed by special processes; thus, tryptophan is broken down into indol and its derivatives, arginine into creatinine, and cystine into taurine.

The fate of the ammonia is not known exactly, two theories being current. The classical view was that the liberated ammonia immediately combined with carbonic acid, and that urea was derived from the ammonium carbonate thus formed. Some do not accept this hypothetical process, and believe that the production of urea occurs by direct oxidation of proteins, without passing through the stage of ammonia.

The earlier view thus postulated the liberation of ammonia

by deamination leading to the formation of ammonium carbonate and carbamate, from which, by dehydration, urea was formed. It followed from this that the circulating blood should contain ammonia salts, and this blood ammonia was the origin of the urinary ammonia. Now, the most delicate analysis has thrown doubt on the existence of ammonia in the circulating blood. It would seem, rather, that the blood contains an ammoniogenic substance, capable of being transformed into ammonia only outside the blood-vessels, and that the kidney is the chief organ where ammonia is formed.

On the other hand, the experimental work of Fosse has shown that urea can be formed in vitro by the oxidation of proteins or of their biological derivatives or of fatty or sugar compounds, in the presence of salts of ammonia. From this, we can assume an analogous oxidation of amino-acids in vivo with production of urea without deamination. This very general process can occur not only in the liver, but also outside of it. This theory, however, does not exclude the possibility of the formation of urea by the liver from ammonia salts of alimentary origin.

It is generally agreed however, that urea is the ultimate product of protein disintegration in man, and that the liver is

the chief seat of origin of urea. Experimental removal of the liver in animals leads to the cessation of urea-formation.

Urea is thus a waste product which is continually entering the circulation and eliminated by the kidneys without being retarded by any threshold of excretion. In certain conditions, elimination may also take place by the digestive tract.

In addition, an appreciable amount of ammonia is eliminated daily in the urine. As mentioned above, it was formerly thought that this ammonia was formed in the liver by deamination of amino-acids, a portion escaping ureogenesis, and, neutralising excess of acid in the blood reaches the kidneys to be excreted in the urine. Thus, the urinary ammonia was thought always to originate from the blood ammonia.

Clinical and laboratory experience do not confirm this hypothesis. In severe hepatic insufficiency, while there occurs an increase in the amino-acids of the blood and urine, with a diminution in urea, no large quantity of ammonia in the blood can be detected, as would be expected if the urinary ammonia had a hepatic origin. Unfortunately, the estimation of ammonia in the blood requires a very delicate technique,

which may explain the divergence of results recorded by different observers. Some biochemists deny the presence of any ammonia in the blood, while others affirm the presence of a minute quantity not exceeding one milligram per 100 c.c. If this ammonia were really present, one would expect it to be increased in the following conditions:

1. In renal conditions which prevent the excretion of ammonia salts, assuming that this does not take place by the digestive tract.

2. In hepatic lesions, interfering with ureogenesis.

3. In conditions of acidosis, when the neutralising bases are being used up.

An increase in blood-ammonia has not been demonstrated in the two former conditions, while in acidosis, neutralisation occurs by the alkali reserve of the blood. Another important point is that there is no relation between the blood ammonia and the urinary ammonia.

All the above facts are in favour of the renal origin of the urinary ammonia, but it is not known from which substance the kidney forms ammonia. On the other hand, the factors influencing ammonia excretion are well-known.

The excretion of ammonia is bound up with the maintenance of the acid-base equilibrium. This is the fundamental fact which explains the variations in the urinary ammonia following the ingestion of food. Herbivora excrete only a small amount of ammonia in the urine; the opposite is the case in the carnivora. This is because the herbivora find in their food a large quantity of bases with which to neutralise the organic acids and acid salts formed in the course of the decomposition of the food-substances. The carnivora, on the other hand, require ammonia to neutralise the excess of acidity brought by the food. In the blood, it is not the ammonia, but the alkali reserve, represented chiefly by the bicarbonates, which neutralises the waste acids. The salts thus formed reach the kidney, where they are decomposed. The basic radicle re-enter the blood-stream, while the acid radicle is eliminated as such, or else is neutralised by ammonia formed in situ. In this way the excretion of acids is assured without decreasing the alkali reserve. Neutralisation by ammonia takes place chiefly in the case of organic acids.

Thus the urinary ammonia appears to be a function of the acidotic tendency of the organism. Now, the liver plays a

preponderating role in acidosis. The combustion of ketonic acids arising from amino-acidolysis takes place chiefly in the liver, and requires the presence of glucose or glycogen. The suppression of this ketolytic function causes ketosis, so that the study of the urinary ammonia can, indirectly, give information on hepatic function. Experimentally, however, partial or total hepatectomy does not cause acidosis, the latter appearing to arise from toxic products liberated by the damaged hepatic cells. This conception seems to be in accord with some recent work on ketolytic functions, according to which the destruction of ketone bodies is accomplished by the muscles and kidneys and not by the liver.

Pathological Considerations:

As noted above, the greater part of the nitrogen of decomposed proteins is transformed by the liver into urea, and eliminated in this form by the kidneys. In pathological conditions, the decomposition of proteins is more or less impaired, a smaller proportion of the protein nitrogen is changed into urea, while the non-urea nitrogen is increased. Experimentally, the same conditions are found. In the

hepatectomised dog with an Eck fistula, according to the technique of Mann and McGath, estimations in the urine have shown a diminution in the urea, an increase in the amino-nitrogen, and the presence of uric acid. Similar conditions are found after acute poisoning of the liver in animals.

The estimation of the different nitrogenous bodies in the blood and urine is thus a means of studying the ureogenic and amino-acid functions of the liver.

NITROGEN AND AMMONIA CO-EFFICIENTS OF THE URINE.

The mere estimation of urea or ammonia in the urine gives no information on the ureogenic functions of the liver, as the amount of waste nitrogen is subject to large physiological variations such as the influence of diet. Greater accuracy is obtained by a study of the relations existing between the different nitrogenous bodies in the daily urine, in a subject submitted as long as possible to a known regime.

URINARY NITROGEN RATIO: $\frac{\text{Urea N}}{\text{Total N}}$

Normally, this ratio varies with the diet, being from 0.82 to 0.84 on a mixed diet, and 0.75 on a vegetarian diet. In pathological conditions of the liver the ratio is lowered.

This ratio would be of great value, if all the nitrogen were ureifiable. Even under normal conditions, this is not the case. This non-ureifiable nitrogen originates chiefly from the disintegration of endogenous protein, leading to the production of creatinine, creatine and purine bodies. The

urea originates chiefly from the amino-acids of exogenous protein. Under certain conditions, for example during a fast, the former process increases, while the latter decreases, and the nitrogen ratio is changed without hepatic involvement. Thus, not much reliance can be placed on this ratio.

COEFFICIENT OF IMPERFECT UREOGENESIS.

(Maillard)

$$\frac{\text{Ammonia N}}{\text{Urea N + Ammonia N}}$$

The nitrogen ratio includes in the denominator the non-ureifiable nitrogen. To avoid this source of error, and starting with the assumption that all the ammonia nitrogen is ureifiable, Maillard established the above coefficient. It is related to the following one, of Lanzenberg,

COEFFICIENT OF ACIDOSIS (LANZENBERG)

$$\frac{\text{Ammonia N + Amino-acid N}}{\text{Urea N + Ammonia N + Amino-Acid N}}$$

and also to Derrien-Clogne's Index,

$$\frac{\text{Ammonia N + Amino-acid N}}{\text{Hypobromite N}}$$

Normally, all these ratios vary with the food, averaging about 6% on a mixed diet, 5% on a vegetarian diet, 4% on a milk diet, and rising to over 10% during a fast.

Pathologically, in cases of hepatic insufficiency, the

ratios tend to become established about 7%.

It was formerly thought that these ratios could reveal imperfect ureogenic power of the liver, since it was considered that the urinary ammonia and amino-acids were ureifiable, and hence an increase in their amount should reveal hepatic insufficiency. However, the urinary ammonia is now thought to be of renal origin, and related to acidosis.

This new conception, however, does not take away entirely the value of these ratios in the evalution of hepatic function. Thus, in severe hepatic insufficiency, they reveal deficiency in the ketolytic function of the liver. Nevertheless, the cases encountered clinically are those of mild hepatic dysfunction, besides which the wide limits of physiological variation render the problem still more complicated.

In order to establish a fixed basis of comparison, an attempt has been made by taking into consideration the Ph of the urine. By this method, the variations due to diet and other physiological causes are narrowed down. This, however, may introduce new sources of error, since it has been shown that urines with a similar Ph can have very different total acidities.

REDUCED AMMONIA COEFFICIENT.

(Hesselbach)

Hesselbach has studied the relation existing between the ammonia coefficient and the Ph of the urine, and has summarised his conclusions in the following formula:

$$Ph \times \frac{\text{Ammonia N}}{\text{Total N}} = K,$$

and he proposes, as a method of testing hepatic function, the estimation of his reduced ammonia coefficient, corresponding to a conventional Ph of 5.8.

Schroeder has suggested the following formula for the estimation of the reduced ammonia coefficient:

$$\frac{\text{Ammonia N}}{\text{Total N}} + \frac{Ph - 4.2}{1.6}$$

CORRECTED AMMONIA COEFFICIENT.

(Feissinger and Guillamin)

$$C.\ A.\ C. = \frac{\frac{\text{Formol N}\ (Ph - 4.2)}{\text{Hypobromite N}}}{1.6}$$

(Formol N is the ammonia N + amino-acid N obtained by the formol method.)

Method:

The 24-hour urine, which has been preserved from fermentation by the addition of mercuric cyanide, is collected. The urea is estimated by the hypobromite method, and the ammonia by the formol method. The Ph is measured by colorimetry or electrometry.

Results:

Normally, on a mixed diet, the corrected ammonia coefficient varies from 4.5 to 6.

In pathological conditions of the liver, it may rise above 12. A progressive increase in the value of the coefficient always indicates a bad prognosis.

A modification of this test is performed in the following way. The early morning urine is collected, and then 150 gm. of honey are swallowed, with 2 glasses of tea. The urine is collected 2, 4, and 6 hours later. The corrected ammonia coefficient is determined for each specimen, and the results expressed graphically.

Results:

Normally, slight variations and irregularities occur, from 4 in the first specimen to 6 in the last.

In slight hepatic insufficiency, the curve falls during the 2nd hour, and rises again in the 4th hour. In severe hepatic insufficiency, the first value is raised, and the curve rises progressively from 8 or 10 to 14 (70).

$\frac{\text{CHOLESTEROL}}{\text{RESIDUAL N}}$ COEFFICIENT OF THE BLOOD.

(Redmond & Colombe)

The estimation of the above coefficients are attempts to measure the residual acids arising from the decomposition of proteins. But any acidosis of extra-hepatic origin constitutes a source of error which must first be eliminated. While bringing greater accuracy to the study of acidosis, these coefficients do not always give satisfactory results, because they do not take into account several important factors regulating ammonia excretion, the Ph of the kidney capillaries, the quantity of ammoniogenic substances in the blood, and the total acidity of the urine. So long as we are ignorant of the physiological laws which regulate the excretion of ammonia, the study of these ammonia coefficients cannot give precise information on the condition of the liver. Clinically however, the estimation of the Corrected Ammonia Coefficient, or of Maillard's Coefficient is of great value, provided that renal excretion is unimpaired.

The residual introgen is the difference between the total nitrogen and urea nitrogen of the blood. The chief difficulty lies in estimating the total nitrogen of the blood, the methods for which are elaborate and lack precision.

In the serum, the average value of the coefficient is between 18 and 16. In whole blood, oxalated or fluorated, it varies from 8 to 6.

In conditions of nitrogen retention, the level of the residual nitrogen is generally raised, the cholesterol also being raised in favourable cases, and where the liver is not involved, but falling in severe cases, or where there is a hepatic lesion.

In favourable cases, the coefficient in the serum is slightly lowered (12 to 11), and greatly diminished in severe cases (7 to 5).

In whole blood, the prognosis is still good when the coefficient is between 6 and 5, but becomes grave when it falls to 4 or 3.5.

The above figures have still to be confirmed, and their exact significane worked out before they are generally accepted.

AMINO-ACID TEST.

The test should be carried out on a 24 hours specimen of urine, and on a known diet. The amino-acids are estimated colorimetrically or by the formol method.

Results:

Normally the amount varies with the diet, being 10-35 cg. on a meat diet, and 0-10 cg. on a vegetarian diet.

In hepatic conditions, the amino-acid nitrogen is increased, being 15-30 cg. in icterus gravis.

In addition, Bith's ratio,

$$\frac{\text{Amino-acid N}}{\text{Urea N}}$$

is useful. Normally, this varies between 0.5% and 3%. It may rise to 6% in severe hepatic insufficiency.

DEAMINATION BLOOD INDEX.

(Feissinger, Olivier and Herbain)

Method:

The principle of the method is that trichloracetic acid in concentrations below 30% precipitates proteins, but allows intermediate nitrogenous compounds to pass through, while phosphotungstic acid precipitates both proteins and intermediate nitrogenous compounds. The deamination index is

$$\frac{\text{Polypeptide N}}{\text{Total Non-Protein N.}}$$

This ratio allows one to study the disintegration of polypeptides.

A sample of the fasting blood is taken 2 hours after the ingestion of 40gm. peptone. One half of the serum obtained is precipitated by 20% trichloracetic acid, the other half by a solution of phosphotungstic acid.

The nitrogen in the filtrates is then estimated, and

the deamination index is given by the formula:

$$\frac{\text{Trichloracetic filtrate N - Phosphotungstic filtrate N}}{\text{Trichloracetic filtrate N}}$$

Results:

Normally, and in conditions which do not affect the liver, the index varies between 0.10 and 0.15.

In hepatic affections the index is raised, reaching 0.25 in the cirrhoses, and 0.50 in icterus gravis.

The index gives normal results in renal conditions, where, however, the residual N is affected.

The technique of the method is very complicated, preventing its general adoption, but the results obtained are very promising (71).

Conclusions:

All the methods summarised above for estimating the efficiency of the liver, have this common defect, that they give positive results only in advanced cases of chronic disease, and in acute intoxications and infections. In mild and early cases, in which they could be of most value, negative or anomalous results are obtained. Again, in some of the tests,

e.g. the blood deamination index, the technique employed is so complicated as to preclude their use in current practice; in others, e.g. the estimation of the total N of the blood or urine, the methods are unsatisfactory and inexact, and different results are obtained from different techniques.

On the whole the results of this series of tests are disappointing at present, and they are not recommended for systematic investigation of hepatic function.

WIDAL'S TEST - HAEMOCLASTIC CRISIS.

This test was elaborated by Widal, who momentarily established an anastomosis between the portal vein and inferior vena cava of a dog, during the digestive period following a meal (72, 73, 74). A typical "crise hémoclasique" developed. A similar result was obtained after injecting commercial peptone into the vein of a dog.

The haemoclastic crisis comprises the following phenomena.

1. Leucopenia.
2. Reduction in the number of blood-platelets.
3. Prolongation of coagulation time of the blood.
4. Increase in refractive index of the blood.
5. Increase in sedimentation - rate of red cells.
6. Fall in blood pressure and pulse-rate.

In practice, the white cell count alone is taken into account.

Method:

A five hours fast at least is essential, so that the test is most conveniently done before breakfast. A white cell count is made, and then seven ounces of milk are given to drink (the exact amount is not essential). The white count is repeated every twenty minutes for two hours.

Normally, an increase in the blood count of 3,000 occurs, the increase taking place in one hour (75). In order to give a positive result, the count must either remain stationary or else fall.

After a protein meal, proteoses and peptones - the result of incomplete digestion of proteins - enter the portal circulation, and reaching the liver, are broken down into amino acids, and these are then either metabolised by the liver, or else enter the general circulation to be utilised by the body tissues.

Where the hepatic function is deranged, the liver is unable to cope with the products of intestinal protein digestion, and proteoses and peptones pass through the liver, and enter the general circulation. It is thought that their presence upsets the normal colloidal balance of the blood, and gives rise to the

series of phenomena known as the haemoclastic crisis (76).

Unfortunately, in practice it has been found that the test is positive in many conditions other than those directly affecting liver function. Thus it has been shown that digestion leucopenia is normal in children under ten years of age (77), and that a positive reaction is present in 33% of cases of normal pregnancy.

A positive result has been obtained in such conditions as appendicitis, tuberculous peritonitis, malaria (78), asthma and epilepsy (76).

Zehnter obtained a negative result in 50% of his hepatic cases, while other investigators report a positive result in 50% of their non-hepatic cases (78).

It is obvious from these findings that the liver cannot be the only factor concerned in the production of the haemoclastic crisis, and until these are known, the real significance of the phenomena must remain unknown to us.

It has even been questioned whether the test is a true liver function test at all, and it is held by some that the crisis is due to overtone of the vagus and sympathetic nerves (79, 80) but this is an extreme view.

Since a positive result is obtained in so many different conditions, and a negative result does not exonerate the liver, it must be admitted the test is of no great value, and too much reliance should not be placed on it.

THE LYON-MELTZER TEST.

This test enables the bile to be withdrawn and examined directly for abnormal constituents. It depends on the fact, first noted by Meltzer (81) that the application of a 25% solution of magnesium sulphate to the duodenum causes contraction of the gall-bladder, and a flow of bile into the duodenum.

Method:

The test is performed on a fasting stomach. The patient rinses his mouth with a good antiseptic solution, say, potassium permanganate 1 gr. to 2 ounces of water, followed by a rinse with a weak solution of zinc chloride. A sterile duodenal tube is then passed into the stomach; the fasting gastric residuum is aspirated into a sterile vessel, measured, and grossly observed for consistency, mucus, etc. and then cultivated and studied chemically for acidities, bile and occult blood. Microscopical examination is also carried out. The stomach is then thoroughly rinsed, and the patient is given a glass of

water to drink while slowly swallowing the tube to the duodenal point, the while lying on the right side, with a pillow or sandbag elevating the hips.

The tube usually passes into the duodenum within fifteen to forty-five minutes. When it has done so, the fact can be determined by the duodenal "tug", the character of the aspirated fluid, and the failure to recover immediately by vacuum aspiration, material (water, broth, etc.) which the patient swallows by mouth. Difficulty is occasionally encountered in entering the duodenum because of vagotonic states, or local pylorospasmic states (a reflex from duodenal ulcer, cholecystitis or chronic appendicitis) which can be overcome by an injection of atropine sulphate or by several days' use of belladonna or benzyl benzoate. Once it is sure that the tube is in the duodenum, a barrelful of air is introduced from a 1 ounce capacity syringe to balloon out the duodenal walls from the metal-tipped tube (to prevent traumatism of the duodenal mucosa); a connection is made with the first sterile aspirating vacuum bottle, and gentle aspiration of the duodenum is begun (82). Or, the tube is allowed to hang over the edge of the bed, and allowed to

drain, by gravity, into a series of test-tubes.

In the fasting duodenal state, and when the physiological condition is normal, the sphincter of the common bile duct should be closed, and the duodenal contents should be bile-free, pearly-grey, of syrupy and stringy consistency, fairly transparent, and should have a relatively small amount of flocculent or flaky sediment. In states of duodenitis, the gross appearance, the microscopic sediment and the chemical tests differ widely from the normal. When bile is found in the fasting duodenum in sufficient quantities to be grossly visible, there is either a disturbed physiological condition, (following the analogy of Meltzer's law of contrary innervation) or a pathological lesion of group organs physiologically related to this intestinal zone.

The first bottle is detached, and with its contents of aspirated fluid set aside for bacteriological, cytological and chemical examination. From 50 to 100 c.c. of a sterile 25% saturated solution of magnesium sulphate are then introduced, by means of a sterile syringe, or by the gravity method, and the tubing connected up to the second sterile aspiration bottle, and gentle aspiration commenced. Usually, within from two to ten minutes, bile begins to be recovered, staining light yellow

the magnesium sulphate solution still in the duodenum. When the colour deepens to a pronounced yellow, the material already collected in the second bottle is decanted into a sterile glass container; the bottle is re-attached, and biliary drainage is continued. Other methods of inducing a flow of bile are the installation of olive oil, or the injection of 2 c.c. of pituitrin, which causes a powerful contraction of the gall-bladder.

The bile flows intermittently, somewhat after the manner in which urine is secreted from the ureters into the bladder in patients in whom cystoscopy and catheterisation of the ureters have been performed. Especially is this intermittent flow observed after the bile already in the ducts and in the gall-bladder has been drained, and bile is being collected as it is secreted from the liver capillaries. The first bile aspirated is the bile present in the bile-duct - probably only the common duct - because it is lighter yellow, more likely to be transparent, and much less mucoid than the bile seen later. This first bile may be from 10 to 20 c.c. in amount, and may require from one to three minutes to aspirate, when a sudden transition appears (seen first in the glass window

of the tube) and the bile becomes darker, more viscid and more concentrated, and, in normal gallbladders, remains transparent, but is more of a molasses yellow. This type of bile is that stored up in and delivered from the gall-bladder, and when it appears first in the glass window, the second sterile bottle is detached and replaced by a third sterile collecting bottle into which the bile is allowed to flow until all of this darker bile (more viscid, transparent, or turbid) has been collected, and is being replaced by a lighter yellow, thinner, and usually transparent bile which is aspirated much more slowly and intermittently, and which is bile freshly secreted from the liver. When this second transition appears, the third bottle is detached, and a fourth sterile bottle attached to collect the liver bile.

Each sample of bile thus collected is examined chemically, bacteriologically and cytologically. The bile from the gall-bladder varies in amount from 30 to 100 c.c. The highest normal should not exceed 75 c.c. The variation in amount depends on the length of time since the gall-bladder last emptied itself, partially or completely, in response to normal digestive needs; on mechanical factors that prevent its ready

emptying of itself unassisted, such as inflammatory adhesions, or thick, viscid, sluggishly flowing bile caused by catarrhal conditions of the gall-bladder; or the variations may be dependent on the tonicity of the gall-bladder musculature, for it is readily conceivable that in over-distended, dilated gall-bladders the muscle-wall has been so stretched, as to possess insufficient tone to propel the contents easily enough through the cystic and into the common duct.

The three samples of bile are analysed for their content of bile pigments, bile salts, and cholesterin.

Estimation of bile-pigments in the bile by Van den Bergh's Method:

In a centrifuge tube are placed 2 c.c. of bile and 4 c.c. alcohol, which are mixed and centrifuged. 2 c.c. of the centrifuged fluid, 1 c.c. of alcohol and 0.5 c.c. of the diazonium reagent are then placed in one cup of the colorimeter and in the other is placed the iron rhodanate or cobalt standard. A comparison is made, and the reading multiplied by 5. The result is given in Van den Bergh units, the standard corresponding to a 1 in 200,000 solution in bilirubin, or one Van den Bergh unit.

Estimation of Biliary Salts in the Bile:

The following solutions are required.

1. 25% solution of basic lead acetate, filtered, and kept tightly corked to prevent admission of air.

2. 95% alcohol.

3. Concentrated acetic acid.

4. 1% solution of furfurol in absolute alcohol.

5. Concentrated phosphoric acid, specific gravity 1.71 to 1.75.

6. Standard solutions of cholalic acid in 80% alcohol.

Standard I.	.2%
II.	.1%
III.	0.05%
IV.	0.025%
V.	0.0125%

A metal water bath is required, in which can be placed a small wire container which will hold the various test-tubes required.

The bile is diluted with its own volume of water, and 2 c.c. are placed in a graduated test-tube, 0.5 c.c. lead acetate solution added, and well-mixed. 95% alcohol is then added to the 10 c.c. mark with constant shaking, and the

contents filtered into another test-tube.

1 c.c. of the filtrate is measured into a graduated test-tube, and to 5 other test-tubes are added 1 c.c. of the 5 standard solutions of cholalic acid.

To each are added 0.5 c.c. of the furfurol solution, and phosphoric acid to 5 c.c. The contents are mixed by shaking without inverting the tubes.

The tubes are placed in the wire container, kept in the boiling water bath for 5 minutes, then placed in a cold water-bath for another 5 minutes.

Acetic acid is added to 10 c.c. and mixed thoroughly by inverting the tubes.

After a few minutes, the unknown is compared with the nearest standard which matches, in a Dubosq colorimeter.

The result is multiplied by 2, since the bile was originally diluted (83,84).

Estimation of Cholesterine in the Bile:

The standard solution is made as follows: Into a graduated test-tube pour 5 c.c. of a 0.06% solution of cholesterine in chloroform. Add 2 c.c. of acetic anhydride

and 5 drops of sulphuric acid, mix, and wait half-an-hour before making a reading.

2 c.c. of bile are placed in a Grigaut cholesterimeter (a long tube with stop-cocks to allow fluid to be withdrawn from the bottom of the tube). 20 c.c. ether are added, and well mixed. Two layers separate out. Decant the bottom layer. Then wash the ether by adding about 15 c.c. distilled water, allow to stand for a few minutes, then decant the water. Wash the ether a second time and again carefully decant the water.

Empty the tube into a porcelain basin by pouring the ether through the top end of the tube. Rinse the tube with a few c.c. of ether, and again pour into the porcelain tube, which is then placed in a water bath till the ether evaporates.

Chloroform to 5 c.c. is added, shaken up, and the whole poured into a graduated tube. 2 c.c. acetic anhydride and 5 drops of pure sulphuric acid are added and mixed. Wait half-an-hour before comparing with the standard, after which time, the green colour obtained does not vary in intensity.

Compare with the standard before a ground-glass background.

If the tubes match, the 2 c.c. of bile contain the same

quantity of cholesterine as the standard, i.e. 0.003 gm. or 1.5 gm. per litre.

If the tubes do not match, proceed as follows: Withdraw fluid from each tube till 5 c.c. are left. Then add to the deeper coloured tube sufficient chloroform till a match is obtained. Alternatively, a mixture of chloroform, acetic anhydride and sulphuric acid may be added, but this is unnecessary. The concentration of cholesterine can then be easily calculated (85).

Results:

In choledochitis, the bile first collected is pathological. It is more viscid, with an excess of flaky mucus, is usually turbid and "off" colour; it contains pus cells enmeshed in mucus, epithelial cells, and occasionally red blood corpuscles. Cultures may show pathogenic organisms. Choledochitis may be present alone in simple catarrhal jaundice.

In cholecystitis without choledochitis, the first bile collected is relatively normal, but the second bile is grossly pathological. It is more viscid than normal gall-bladder bile,

and turbid with a flaky or stringy mucus; pus cells, red-blood cells and desquamated epithelium are present, and cultures show pathogenic organisms. The colour varies from a deep golden yellow to various shades of green or greenish-black.

In cholelithiasis, evidence of cholecystitis is present but in addition, the bile may contain a sediment, gritty or sandlike in consistency, and microscopically consisting of crystals of bile-salts.

Results of chemical examination of the normal bile:

1. Bile pigments.

Bile A	12 units
Bile B	50 units
Bile C	6 units

2. Bile salts.

Bile A	2 - 4 gm. per litre.
Bile B	10 - 14 gm. per litre.
Bile C	1 - 2 gm. per litre.

3. Cholesterin.

Bile A	0.25 gm. per litre.
Bile B	1.10 gm. per litre.
Bile C	Traces.

In catarrhal jaundice, while the pigment content of the bile remains high, the salts are greatly diminished in amount. This may be due to decreased production of salts, or to tissue-fixation, or to transformation into other compounds.

In cholelithiasis, if the cystic duct is occluded, no bile B is obtained. If the cystic duct is patent; the results obtained are highly characteristic. The bile pigment concentration is normal. That of cholesterin and bile salts, however, is greatly diminished in all three samples of bile, i.e. dissociation is present.

In atony of the gall-bladder, the results are again typical. A very abundant and very dark B bile is obtained, in which all the constituents are greatly increased, the pigment concentration being doubled, while the salts may reach four times the normal amount.

Conclusions:

Duodenal intubation is of very limited application, its value being confined to infective and inflammatory conditions of the biliary tract. It is of use in the diagnosis of complete biliary obstruction, in which condition no bile is obtained, and occasionally in the differential diagnosis between cholelithiasis

and cancer of the head of the pancreas.

From the point of view of prognosis, the test is of no value whatever.

UROBILIN AND UROBILINOGEN TESTS.

Very conflicting views are held on the subject of urobilinogen formation, and the complete chapter of its origin and fate in the body has yet to be written. It is generally accepted, however, that the bilirubin of the bile which enters the intestines is converted into a pigment, stercobilin, identical with urobilinogen. This change is brought about by the action of bacteria. The stercobilin is absorbed into the circulation, and reaches the liver as urobilinogen. Thus, a certain amount of urobilinogen is normally present in the blood. The pigment is abstracted by the liver and built up again into bilirubin, but a small amount is excreted by the kidneys, and traces are normally to be found in most urines. On standing, the urobilinogen is converted into urobilin by a process of oxidation, urobilin not being excreted as such by the kidneys. This change can also be brought about by the addition of oxidising substances to the urine, e.g. iodine.

This view of the origin of urobilinogen is the one most generally held, but many observers do not agree with this, and Whipple

and Hooper state (45) "There is not a shred of evidence to indicate that stercobilin is ever absorbed from the intestine".

This is not the only method of formation of urobilin, according to some. Kalm holds that a diseased liver may form urobilin either directly as a product of its cells, or indirectly from decomposition of bilirubin within its bile passages.

In liver disease, the amount of urobilinogen not picked out by the hepatic cells increases, and, accumulating in the blood, is excreted by the kidneys in easily demonstrable amounts. Again, in haemolytic conditions, increased bile-formation by the liver occurs, and the liver is unable to deal with the circulating urobilinogen. A relative insufficiency of the liver is thus present, and the urobilinogen, increasing in the blood, is excreted in the urine. Another factor may be the presence of liver damage in haemolytic conditions, as it is probable that injury to the hepatic cells occurs in diseases accompanied by increased blood-destruction.

The occurrence of putrefactive conditions in the intestines also leads temporarily to increased urobilinuria but this condition does not last long.

French workers (86) hold that the presence or absence of

urobilin in the urine depends on the amount of bilirubin in the blood, the kidney threshold being higher for bilirubin than for urobilin. They state that when the amount of bilirubin in the serum reaches its kidney threshold, it is excreted unchanged, but when it falls below this point, urobilin is again formed by the tissues, and is excreted as such. So, in mild jaundice we get urobilinuria, and in severe jaundice, bilirubinuria. This view is not generally held, and the facts are capable of other explanations.

In conditions where bile is unable to reach the intestines, a small amount of stercobilin may still be found in the faeces. This is probably due to the excretion of bilirubin into the intestines, a bilirubinaemia being present in these conditions.

UROBILIN TEST.

This test is based on Schlesinger's observation that if an alcoholic solution of zinc chloride or acetate is added to a urine containing urobilin, a green fluorescence develops. By finding the greatest dilution of the urine in which this fluorescence can be observed under given conditions, an estimate of the amount of urobilin may be made. The urobilinogen is first converted into urobilin by the addition of iodine. (87)

Method:

10 c.c. of urine are measured, into test-tube A, and to this are added three drops of a 5% alcoholic solution of iodine. After shaking up, 1 c.c. of this is measured into test-tube B. I. To test-tube A, which now contains 9 c.c. of urine, are added 9 c.c. of absolute alcohol and 1 gm. of zinc acetate, and after complete solution of the zinc acetate, the contents are filtered through a plain filter (9 c.m. in diameter) into a test-tube having a width of 16 m.m. (Dilution of urine is 1/2).

II. If the filtrate fluoresces, to test-tube B are added 3 c.c. of water, 1 c.c. of a clear, 20% solution of zinc acetate in water, and 5 c.c. of absolute alcohol. After mixing, it is filtered. (Dilution of urine is 1/10).

III. If the filtrate fluoresces, 5 c.c. of the undiluted urine are measured out. To this are added 5 c.c. of water, and two drops of solution of iodine. From the mixture, 1 c.c. is measured into another test-tube, and mixed with 3 c.c. of water, 1 c.c. of 20% zinc acetate solution and 5 c.c. of absolute alcohol, and then filtered. (Dilution of urine is 1/20).

IV. If the filtrate fluoresces, 5 c.c. of urine are measured into 15 c.c. of water, and two drops of solution of iodine added. From the mixture, 1 c.c. is measured, and the procedure is the same as in III. (Dilution of urine is 1/40).

The filtrate is always examined for fluorescence in daylight falling from behind the observer through a test-tube 16 m.m. wide. Ammoniacal urines ought always to be acidified with acetic acid before making the test. In the case of highly acid urines, it is advisable to make alkaline with ammonia, and then to acidify with acetic acid.

The urine of many normal persons contains urobilin in

sufficient quantity to give this test, but only rarely will a normal urine give a positive reaction in a dilution of 1/10. The lowest limit for a pathological urobilinuria may therefore be taken as 1/20. Positive results in patients suffering from liver-disease have been obtained in urine-dilutions of 1/80, and even in greater dilutions.

Some technical disadvantages to the test are: (a) The variability of the fluorescence, the intensity varying with fine shades of difference in the reaction, thus often interfering with the end result.

(b) The conversion of urobilinogen into urobilin, by reducing agents such as iodine introduces an element of uncertainty as to whether the entire urobilinogen has been thus changed. The sunlight always causes some loss in the urobilin.

(c) Some loss of urobilin when present in large quantities also takes place in the precipitation with the alcoholic solution of zinc acetate.

UROBILINOGEN TEST.

This test is dependent on Ehrlich's aldehyde reaction, and consists of a series of dilutions of the urine carried to a point where no further reaction takes place. The end-result is read off where the faintest pink is still discernible. The reading is made by looking through the mouth of the test-tube, holding the tube obliquely against a white background. Dilutions may be made with plain tap water (88).

Ehrlich's aldehyde reagent consists of 2 gm. of dimethyl-amidobenzaldehyde in 100 c.c. of 20% HCl solution). 1 c.c. of Ehrlich's reagent is added to the whole urine, and the strength of the reaction noted by the rapidity and intensity of its development. The full development requires from 1 to 3 minutes. In strong urobilinogen concentrations, a deep red colour comes on promptly. The presence of abnormal quantities of urobilinogen is easily gauged from this qualitative test, and dilutions are not proceeded with if the colour remains a light red within the time allowed, as here one deals with normal values.

If, however, the original reaction indicates an increase, then a series of dilutions are made by adding 1 c.c. of the urine to 20, 30, 40, 50, 100, 200 c.c. of water, or more. From 10 to 15 c.c. of each dilution is placed in test-tubes, and to each is added 1 c.c. of Ehrlich's reagent. The reading is made after from 3 to 5 minutes, so as to allow the full development of the colour. The last dilution must be the faintest pink discoloration, and the quantitative determination is expressed in terms of the greatest dilution of the urine in which the pink colour is present e.g. 1:10, 1:50, etc. If the colour does not appear within 5 minutes, it may be disregarded. If the tap water is too cold, the reaction may be somewhat retarded. Care should be taken that the colour is a genuine pink and not a yellowish brown, as is often found in urines containing bile, or in concentrated urines. The test is preferably carried out in daylight. It is best not to make a reading in the bright sun, as the shining rays have a tendency to intensify the colour, Artificial light also does this to some extent.

This test disregards entirely the estimation of urobilin, as this substance is never found in the freshly-voided urine, and forms only very slowly if the urine is kept away from

sunlight. If urines containing known amounts of urobilinogen are allowed to stand for 24 hours away from the strong light, and in the cold, there is a loss, after 24 hours, of one fifth of the total urobilinogen. If the exposure is continued for another 24 hours, there is a loss of another fifth, and so on, approximately one fifth or slightly less for every 24 hours.

Single fresh specimens should be examined, rather than 24 hour specimens, as in this way, one specimen may be found with a high urobilinogen content. Such an increase, if occurring even once a day, signifies an existing pathological condition.

This test can be adapted to the estimation of the amount of urobilinogen in the stool. Difficulties present themselves owing to the presence of indol and skatol, which give a similar reaction with Ehrlich's aldehyde reagent, and show similar spectroscopic absorption bands in alcoholic extractions. It is therefore necessary first to remove the putrefactive substances. This is best done by rubbing the stool in a mortar with petroleum ether, and centrifuging. Several such extractions are made until the clear supernatant petroleum ether no longer gives Ehrlich's reaction. Of the remaining faeces, alcoholic extractions are made till all the urobilinogen is

taken up. Using a definite weight of faeces, and a definite amount of alcohol, the urobilinogen can be quantitatively estimated in similar dilutions.

By the above method, urobilinogen is normally found to be present in the morning urine, but in amounts which fail to give a reaction if the urine is diluted more than 1 in 10. During the afternoon and evening, the maximum dilution of urine which will react to the test is 1 in 20. The last figure therefore is the largest dilution compatible with normality which will react to the test.

COMBINED UROBILIN AND UROBILINOGEN TEST.

When undiluted urine is examined spectroscopically, nothing is seen, as a rule, except a general absorption of light, which becomes deeper and deeper towards the blue end of the spectrum. Even if urobilin is present, the absorption band cannot be clearly differentiated. When the amount of urobilin, however, is much larger than the other light absorbing substances present, one finds that on simple dilution with water the band between b and F becomes more and more distinct, so that it at last stands out sharply. On still further dilution it begins to fade, and a more or less definite point is reached at which it becomes invisible when the full amount of light is allowed to pass through the spectroscope, but can still just be seen when the light is diminished (89).

It has also been found that when Ehrlich's aldehyde reagent is added to urine containing urobilinogen, a characteristic absorption band is produced between D and E, which lies between the red and the yellow.

The following test consists in diluting a sample of urine till the characteristic absorption bands as seen by the spectroscope are no longer visible when the full amount of light is allowed to enter the instrument, but are still visible when the light is partly shut off.

A twenty-four hour sample of urine is collected in a vessel made of dark-brown glass, and which is kept in the dark during collection of the sample. A few thymol crystals are added to prevent fermentation. After measuring the amount of the twenty-four hour urine, 10 c.c. are mixed with 10 c.c. of a saturated alcoholic solution of zinc acetate, and after a few minutes, filtered. 10 c.c. of the filtrate are taken, and 1 c.c. of Ehrlich's solution is added. The development of the urobilinogen band is not instantaneous, a quarter of an hour elapsing before it reaches its full intensity. It is better to wait for an hour before making the reading, and during this time, the solution should be kept in the dark. Citron's hand spectroscope is the most convenient instrument to use. The filtrate is washed into a graduated tube, and diluted with tap-water until first one and then the other band of light absorption has disappeared when the full amount of light enters

the spectroscope, but is still visible when the light is partly shut off. This gives a fairly definite end-point. It is important that the light should be always of approximately equal intensity, and readings are conveniently made in a dark room with a Tungsten electric bulb, holding the spectroscope close to the source of light.

In highly coloured urines one may be in doubt as to whether or not a trace of urobilin is present, for there may be so much general absorption of light as to obscure the urobilin band in the undiluted filtrate. There is no such difficulty with the urobilinogen band which lies between the red and yellow where there is no marked light absorption. With urines containing bile, if the amount of urobilin is not very large, it is necessary to add some Fuller's earth and to leave the mixture standing for some time before filtration. If this is done, the urobilin band can usually be read, even in the undiluted filtrate.

The dilution required gives the value for 5 c.c. of urine. If this figure is multiplied by the number of 5 c.c. quantities inthe twenty-four hour urine, the number of dilutions which would have been necessary if all the urobilinogen and urobilin

in the twenty-four hour amount had been concentrated in a volume of 5 c.c. is obtained. For instance, in a twenty-four hour urine measuring 1,000 c.c. a reading of ten dilutions for urobilinogen and twenty for urobilin would be 30 x 200 = 6,000.

With the above method, a positive result in a twenty-four hour specimen of urine indicates an abnormal increase over the amount usually present. Only very rarely is there present a faint degree of absorption in the region of the urobilinogen or urobilin bands in the urines of persons in apparent health.

Results of the Tests:

The tests have been found to give positive results in those acute and sub-acute liver diseases in which the parenchyma is involved. In cholecystitis, cholelithiasis, carcinoma of the common bile-duct, and in other chronic biliary diseases, the tests show no alteration in liver function.

In chronic liver diseases, however, contrary to the acute states, a positive test may not be obtained, and in fact, the test is usually negative. Many cases of cirrhosis of the liver, with hepatic and splenic enlargement and ascites fail to show a pathological increase in urobilinogen. In sub-acute liver

diseases, and in the acute exacerbations of chronic diseases, the urobilinogen is usually increased.

In haemolytic diseases, such as pernicious anaemia, haemolytic icterus, septic states, malaria, lead-poisoning, and other haemolyses produced by poisons such as antifebrin and sulphonal, a pathological increase in urobilinogen is obtained.

Other conditions in which the test is positive are: Toxic states, such as eclampsia and toxic pregnancy, the later stages of tuberculosis, intoxication resulting from the intake of large amounts of alcohol, and infectious diseases, such as measles and scarlet fever.

In malaria, increase of urobilinogen is a constant finding, and the diagnosis is thus enabled to be made before the discovery of parasites in the blood.

The constant presence of urobilinogen in the urine in catarrhal jaundice, and its total absence in mechanical icterus, such as is encountered in carcinoma of the head of the pancreas and of the biliary tracts forms a marked distinguishing diagnostic feature between this benign form of jaundice and that due to malignant causes.

In the differential diagnosis between pernicious and other

forms of anaemia, a positive result invariably indicates the presence of pernicious anaemia, as urobilinogen is not excreted in the secondary anaemias.

These tests are therefore very useful clinical aids, but they respond only to the more acute and sub-acute types of liver disease. A positive result is of great value and definite proof that a pathological process is present. A negative result is valueless, and may be obtained even in the presence of very advanced chronic disease.

WATER METABOLISM FUNCTION TESTS.

The water of the body has an endogenous and exogenous origin. The exogenous water is derived from the food and drink; the endogenous water is formed in the course of chemical reactions in the body.

The water of the body can be divided into three systems (Achard).

1. The water of the tissues or water of constitution.
2. The water of the blood, or water circulating in the blood-vessels and lymphatics, and forming a closed system.
3. The water distributed in the interstices of the cells and tissues and in the serous cavities, the supporting or reserve water.

Normally, the movement of water in the body is regulated by a complicated system which tends to maintain the state of hydration of the tissues and the concentration of the body fluids at a constant level. This regulating system is but imperfectly known, but it is put into play by nervous, mechanical and physico-chemical factors. The passage of water between the

blood capillaries and tissue spaces depend on the intra-vascular pressure, the osmotic pressure of crystalloids, and the osmotic pressure of proteins contained in the blood. Ignoring the crystalloids, the passage of water into and from the tissue-spaces depends on the difference between the capillary blood-pressure, and the osmotic pressure of the proteins. When the former exceeds the latter, transudation occurs, and conversely, when the osmotic pressure of the proteins exceeds the intra-capillary pressure, re-absorption of water takes place. Hydraemia produced by ingestion of a large quantity of water lowers the tension of the proteins, and favours the passage of water into the tissue-spaces; the secretion of urine then diminishes the hydraemia, the concentration of the proteins is thereupon increased, and re-absorption of water takes place.

Govaerts has shown that the albumin of the serum has an osmotic pressure more than four times as great as that of the globulin. A diminution in the albumin-globulin ratio is observed in certain cases of oedema. The lipoids have also been shown to play an important part, as well as the hydrogen ion concentration of the blood. Other factors doubtless are also involved, but they all depend on the integrity and harmonious

functioning of the nervous system, cardio-vascular apparatus, and various glands. The liver, kidney and endocrine glands can act upon and affect the physico-chemical equilibrium in different ways, for example, by disturbing the metabolism of proteins and lipoids.

Pathologically, the liver can affect water metabolism in the following ways:

1. Mechanically, by obstructing the portal circulation, and thus the absorption of water.

2. By causing physico-chemical alterations in the blood, e.g. by disturbances in the metabolism of proteins and lipoids.

3. By causing lesions of the endothelium of the capillaries. This, while not actually causing oedema, may yet determine its localisation.

FRACTIONAL DIURESIS TEST.

Method:

The patient is put on a salt-free diet for 2 days. The test is performed fasting, and in the early morning. The bladder is emptied, and the night-urine rejected. He then swallows 200 c.c. of water every half-hour till 800 c.c. have been drunk. The urine is collected every half-hour for three hours, and the amount measured each time. The test is repeated the following day, with the patient lying down, the first test having been performed in the upright position.

Previously to the test being performed the day and night urines are collected and measured. Normally, the day urine should be $\frac{2}{3}$rd of the total urine.

Results:

Normally, the volume of urine passed in the three hours should be equal to, or somewhat more than that of the water swallowed. The urine passed should be the same in the upright

and lying-down position. The curve of elimination rises to a maximum and then falls again, so that the last specimen is approximately equal to that of the first.

In pathological conditions, the following variations are met with.

1. Cardio-vascular conditions: Elimination, which is decreased in the upright position, approaches the normal in the lying down position, the improvement in the latter case denoting the circulatory origin of the disease.

2. Renal Conditions: The total quantity eliminated is more or less diminished. The effect of posture is little, if at all, marked. Excretion tends to become uniform, and is more so, the more marked the lesion. Similarly, the night-urine tends to equal the day-urine.

3. Hepatic conditions, with increase in the portal pressure: There is a considerable delay in excretion, which is not influenced by posture. Renal Conditions, however, must first be excluded. Where several pathological conditions are present, interpretation of results becomes very difficult.

The test is a useful one where there is reason to suspect hepatic congestion or early cirrhosis, but conclusions can only be tentative (90).

CUTANEOUS OEDEMA TEST.

(Aldrich & McClure)

Method:

Two drops of serum are injected into the skin of the front of the forearm. The time taken for the ball of oedema to absorb is then noted.

Results:

Normally, the time of absorption varies from 50 to 90 minutes.

When oedema is present, absorption occurs in a few minutes. In subjects who are not oedematous, but show a tendency to oedema, absorption takes 30 to 45 minutes.

The absorption time is also greatly decreased in conditions of dehydration; the test is therefore only an expression of lack of water of the tissues, and gives no further information (91).

TRANSUDATION TEST.

Method:

A mark is made on the patient's wrist, and he then plunges his hand vertically into a vessel of water up to the mark. The level of water in the vessel is then marked. The hand is withdrawn, and a tourniquet placed round the wrist at the level of the mark previously made, so as to obstruct the venous circulation only, for 10 minutes. The tourniquet is taken off, the hand held horizontally for 5 minutes, then again immersed in the vessel of water up to the mark on the wrist. The level of water in the container is noted, and the difference represents the increase in volume of the hand due to oedema caused by the constriction.

Results:

Normally, the test is negative.

In pathological conditions, the increase in volume may exceed

50 c.c. In hepatic conditions with oedema, or even only with ascites, the transudation test is markedly positive. It may also be positive in grave hepatic affections without either oedema or ascites.

The results obtained by the transudation tests are parallel to those obtained in the cutaneous oedema test, and measure the permeability of the capillary endothelium to transudation of plasma. It is not specific, and is positive in other conditions complicated by oedema (92).

THE BLOOD-COAGULATION TEST.

The coagulation of blood results in the formation of fibrin from the fibrinogen present in the blood by the action of a ferment, thrombin. The latter, however, is not present as such in the circulating blood, but occurs as thrombogen, which is converted into thrombin by the action of two substances, thrombokinase, derived from the blood-platelets and leucocytes, and a soluble salt of calcium.

According to Nolf, all the substances on which coagulation of the blood depend, are present in the blood in the form of a stable colloidal complex. Clotting takes place when the equilibrium of the colloid is disturbed, with the formation of a gel.

It is held, though the evidence is not conclusive, that the liver is the seat of production of fibrinogen, thrombogen and also, anti-thrombogen.

Method:

Blood is drawn into a number of capillary tubes from

a puncture of the finger. The end of each tube is closed with a rubber band, and all the tubes are immersed in a water-bath kept at a constant temperature of 37°C. At the end of each minute after immersion, a tube is withdrawn, and the blood blown out of the tube. When the blood clots, it can no longer be blown out, and the time after immersion when this occurs denotes the coagulation time of the blood.

Results:

Normally, the blood takes 4 minutes to clot, at 37°C. In pathological conditions of the liver, the time is sometimes increased (93).

THE BLEEDING-TIME TEST: (Duck's Test)

Method:

A puncture is made in the lobe of the ear, and the blood removed with blotting-paper every 30 seconds, till bleeding stops.

Results:

Normally, the bleeding ceases after 4 minutes. In pathological conditions of the liver, the time of bleeding is increased.

THE TOURNIQUET TEST:

Method:

A tourniquet is placed round the upper-arm so as to obstruct the venous circulation only, for 10 minutes.

Results:

Normally, nothing happens. In pathological conditions, small purpuric spots appear on the forearm. The test is only positive in severe hepatic insufficiency.

None of the above three tests are of much value, clinically in the diagnosis of hepatic affections.

FIBRINOGEN TEST.

It has been established that the liver is probably the sole source of fibrinogen in the body (94). Working on dogs, whose liver had been injured by chloroform, Whipple and Hurwitz found that the blood fibrinogen showed a drop corresponding to the amount of liver necrosis. By administering sufficient chloroform, the fibrinogen was almost eliminated from the circulating blood, so that bleeding continued for hours from small skin pricks or cuts. As the liver undergoes repair, a process which may be complete in ten days, the fibrinogen reappears in the blood, and, shortly after recovery, the fibrinogen in the blood may reach an abnormally high level.

Method:

About 9 c.c. of blood are collected from a vein into a vaselined syringe, and delivered immediately into a 15 c.c. haematocrit tube, which contains 1 c.c. of a 1% sodium oxalate solution. The tube with its contents is immediately inverted

twice to ensure thorough mixing (95). The mixture is centrifuged at high speed (3,000 revolutions per minute) for 30 minutes. The amount of cellular elements and plasma are read to tenths of a cubic centimetre. Exactly 2 c.c. of the plasma are delivered by a calibrated pipette into a tumbler containing 40 c.c. of a 0.8% solution of sodium chloride, and 2 c.c. of a 2.5% solution of calcium chloride. A similar duplicate sample is set up. The preparations are thoroughly mixed, and allowed to stand at room temperature for 2 hours. To some abnormal blood plasmas, it is necessary to add normal serum, and a longer period may be required to ensure complete coagulation.

The fibrin is freed from the fluid elements by gentle manipulation and pressure with a glass rod. The fibrin mass thus obtained is then washed in distilled water. The clear free fibrin is then placed in a small porcelain crucible, and dried in an oven at 110°C to a constant weight. This requires from 3 to 10 hours, depending on the amount of fibrin. After drying, the crucible is placed in a dessicator, and allowed to cool. The weight of the crucible is recorded to tenths of a milligram. The fibrin is then ignited in the crucible over a Bunsen flame

(15 minutes combustion time). While still warm, the crucible is placed in a dessicator, and when cool, it is reweighed. The difference in the weights before and after burning is the weight of fibrin for the sample analysed. Knowing the weight of fibrin per c.c. of plasma, and the ratio of red blood cells to plasma, we may calculate the amount of fibrin in 100 c.c. of whole blood or blood plasma.

The normal values for blood fibrin in health range from 250 mgm. to 400 mgm. per 100 c.c. of plasma, with an average of about 330 mgm.

No specific results applicable to the diagnosis of hepatic affections have been found. In general, it may be said that diseases stimulating the liver cause an increase in the blood fibrinogen while those which depress the liver lead to a fall in the value. Thus the extent and nature of the tissue injury is the determining factor, and a sterile abscess is as effective in stimulating fibrinogen production, as one of septic origin (96). Nearly all infections give high fibrinogen findings, encephalitis, chronic nephritis, myocardial disease, sepsis, pneumonia, carcinoma and sarcoma. Of hepatic diseases, cirrhosis gives results slightly above and below normal values, cancer and

other tumours give normal or increased values, while congestion of the liver gives uniformly increased figures. Typhoid fever and grave anaemias give low figures.

The only condition which gives consistently very low fibrinogen values is severe liver injury. In the early stages of hepatic disease, the blood fibrinogen is usually normal or increased. This is explained by the large factor of safety possessed by the liver.

It is thus evident that the fibrinogen test is of no value in the clinical diagnosis of hepatic disease.

LIPASE TEST.

Normally, the blood contains a very constant amount of fat-splitting ferment, and in hepatic disease, this is greatly increased. It is supposed, therefore, that the liver inhibits the formation of lipase. Another theory is that the liver deals in some way with lipase absorbed from the intestine, but in its injured state, is unable to do so, and allows the enzyme to pass into the general circulation, increasing its concentration there.

The role of the liver in fat-metabolism appears to be that of converting the saturated fatty acids into the more active unsaturated form for utilisation by the tissues. In some cases of hepatic damage, such as phosphorus or post-anaesthetic poisoning, the fats accumulate in the liver, which is unable to deal with them, leading to a great increase in the size of the organ.

Method:

Into four test tubes 1 c.c. of serum is placed, and 0.3 c.c.

of toluene added to prevent decomposition. 3 c.c. of water are then added to each tube, making the total volume up to 4 c.c. (97).

To two of the tubes, 0.26 c.c. of ethyl butyrate is added, and all the four tubes are incubated for eighteen to twenty-four hours in an incubator at 37°C. The tubes are then removed, and a drop of azolitmin solution is added to each.

The tubes containing serum alone are alkaline, and are titrated with $\frac{N}{10}$ acid. The other tubes, in which the lipase will have produced butyric acid, are acid in reaction, and are consequently titrated with $\frac{N}{10}$ alkali. The amount of lipolytic action is the sum of the amounts of acid and alkali used.

Normally, this is between 0.2 and 0.3 c.c. Values above these figures are excessive, and point to an increase in the amount of lipase in the blood.

This test is a reliable one, a positive result indicating hepatic damage, but unfortunately, the lesions have to be very gross before results of value are obtained, as in chloroform and salvarsan poisoning (98). In fact, it would appear from post-mortem studies, that the only condition capable of increasing the blood-lipase is an acute necrosis of liver cells, and that hyaline or fatty degeneration will not cause this increase.

Liver necrosis, however produced, in a liver previously normal, causes a characteristic rise in the blood-lipase from 0.2 c.c. to 1.2 c.c. or even higher - a rise from five to eight times normal. This rise reaches its maximum after twelve to twenty-four hours, and may remain at this level for twenty-four hours or longer in fatal cases. The curve of blood lipase then falls slowly to normal on the sixth or eighth day, with repair of the injured parenchyma.

Positive results have been obtained in the following conditions: Poisoning due to chloroform, salvarsan, phosphorus etc. Acute yellow atrophy, cholangitis, abscess of liver with considerable destruction of liver tissue, eclampsia, leukaemia, pneumonia, and septicaemia. Cirrhosis of the liver, and other chronic conditions are not associated with an increased blood-lipase.

The test appears to be of very limited application. Only acute conditions, associated with gross liver destruction give positive results and the test is valueless in the chronic and mild types of hepatic disease.

CARBOHYDRATE METABOLISM TESTS.

The carbohydrates of the food are transformed in the digestive tract into monosaccharides, chiefly glucose, and carried by the portal vein to the liver, where conversion into glycogen takes place. The liver can also convert into glycogen the glucose formed from amino acids. The glycogen of the liver, which is the body reserve of sugar, is reconverted into glucose according to the needs of the organism, and used for the production of energy. In addition, glycogen is essential for, or, at least, assists in the performance of various functions of the liver, particularly the combustion of fats, the metabolism of aceto-acetic acid, the formation of urea, and the conversion of creatine into creatinine. Finally, it aids the liver in its antitoxic function by conjugation with toxic substances.

The sugar absorbed from the food, therefore, is partly destroyed and partly converted into glycogen by the liver. The equilibrium between these two processes maintains the

blood-sugar at a constant level. The complex mechanism regulating this equilibrium is not yet fully understood. The utilisation of sugar depends on a glucose-regulating system in which various glands participate (liver, pancreas, suprarenal and hypophysis in particular), the nervous system, and the tissues.

Normally, the fasting blood-sugar is about 0.1%. This represents free sugar - the question of combined sugar or protein sugar is not fully unravelled yet. This average level varies with the age and sex of the individual, and above all, with the diet. Following a carbohydrate meal, there is at first a phase of alimentary hyperglycaemia, followed by a second phase of slight hypoglycaemia. If a second meal is taken shortly after the first, the hyperglycaemia is less than that observed after the first meal. This is called the Staub affect. Again, in subjects submitted to a carbohydrate fast, the hyperglycaemia following a sugar-meal may reach a very high level.

Glycosuria, generally speaking, is a function of hyperglycaemia, without always running parallel to it. Glycosuria may occur without hyperglycaemia, and results from a

lowering of the renal threshold for sugar. This occurs in renal diabetes. Conversely, hyperglycaemia is not always accompanied by glycosuria. This is because the hyperglycaemia does not reach the level of the renal threshold of 0.18%, below which no sugar appears in the urine. The absence of glycosuria in some cases is explained by changes in the kidneys, but this interpretation is sometimes insufficient to explain the phenomena observed.

The following tests describe the methods of estimating the stability of the sugar-regulating mechanism of the body. The part which the liver plays in any observed disturbance must then be determined, either clinically, or by a process of exclusion. On account of the complexity of the factors involved, interpretation is often very difficult.

THE GLUCOSE TOLERANCE TEST.

Method:

The test is performed fasting, preferably in the early morning. A sample of blood is withdrawn, and then 50 gm. of pure anhydrous glucose dissolved in 100 c.c. water are swallowed. 100 c.c. water are given every half-hour, and a sample of blood withdrawn, till the blood reaches the fasting level again. The blood-sugar in each specimen is estimated by Bang's or McLean's method.

Results:

Normally, the fasting blood-sugar is about 0.1%. After ingestion of 50 gm. glucose, it rises to 0.15% in one hour, and regains the fasting level in another hour.

In pathological conditions, modification of the two factors, the level and duration of the hyperglycaemia are observed. Thus, the level may reach 0.18%, but very seldom exceeds it, except in diabetes mellitus. The duration of the hyperglycaemia

may be increased. Thus, while the maximum level is reached after one hour, the fasting level is not regained for two hours or even longer. This delay or duration of hyperglycaemia is more characteristic of sugar-regulating disturbance than the absolute height attained by the blood-sugar concentration.

Positive results are obtained in hepatic diseases, the cirrhoses, icterus, infectious and toxic hepatitis, but also in other affections. Thus, a positive result is the rule in obesity, in which condition the liver is often affected. Graves' disease and hyperpiesis also give positive results. Diabetes must always be excluded, though in this disease the blood-sugar concentration during the test usually exceeds 0.2%, and may last several hours. But all intermediate stages exist between the sugar-regulating disturbance due to hepatic diseases, and that due to diabetes (99). This test is not so reliable as the following ones of carbohydrate tolerance.

THE LAEVULOSE TOLERANCE TEST.

Sugar reaches the liver through the portal circulation in the form of monosaccharides, where it is abstracted and stored as glycogen. The liver, however, is unable to do this with equal facility with all sugars. Thus, the administration of any sugar, except laevulose (and galactose) leads to a rise in the blood-sugar from the fasting level of 0.1% to something like 0.15% falling again to normal in about 1½ hours. In similar doses laevulose does not cause such a rise in the blood-sugar, the increase never being more than 0.02% in normal individuals (100). The inference is that the liver is able to store laevulose, within certain limits, as fast as it enters the blood stream. When, however, the liver is defective, the type of blood-sugar curve is altered, as the liver is unable to prevent the laevulose from entering the circulation (101).

Method:

The patient should fast for at least three hours before the test, after which time the influence of the previous meal on the blood-sugar will have passed, in normal persons. A longer interval still is perhaps better, and a good plan is to instruct the patient to have a light breakfast, and attend for the test 4 - 5 hours later. A sample of blood is taken immediately before the test, the laevulose is then drunk, and further samples are taken at half-hourly intervals, for two hours, the blood-sugar estimations being carried out in the intervals between the taking of the blood. The laevulose is given dissolved in 100 - 200 c.c. water. The amount of laevulose varies with the weight of the patient. The following are convenient amounts (102).

Body-weight	Laevulose
80 Kg.	50 g.
60 Kg.	40 g.
40 Kg.	30 g.

In normal persons, these amounts will cause no appreciable rise in the blood-sugar, but for the purpose of the test, they should not be exceeded, for the following reason. In many

normal individuals, whose blood-sugar shows no rise after 50 g. laevulose, a definite rise can be provoked after giving 100 g. which appears to be beyond the immediate capacity of even a healthy liver.

For clinical purposes, a micro-method of blood-sugar estimation is advisable, in order to avoid venous puncture. The method of McLean is quite suitable (103). In this method 0.2 c.c. capillary blood is taken from a needleprick on the finger or ear into a pipette, with little of no discomfort to the patient, and the estimation can be rapidly and accurately carried out in the intervals between taking the samples of blood.

Generally speaking, the height and duration of the blood-sugar curve are proportional to the degree of liver impairment. Extra-hepatic factors, such as pancreatic disease, diabetes mellitus and endocrine dysfunction have to be excluded before the results of the test are admissible for interpretation, but in these cases, there are usually unmistakable signs pointing to the true nature of the disease.

This test is a very delicate one and there can be no doubt that it estimates very accurately the degree of impairment of

one aspect of the carbohydrate metabolism of the liver. Positive results have been obtained in cases of hepatitis, catarrhal jaundice, malignant disease of the liver, obstructive jaundice due to gall stones, and the jaundice due to salvarsan poisoning. In catarrhal jaundice, the test is positive several days before the onset of jaundice, showing that a hepatitis must be present in this condition. In salvarsan poisoning, the test has been found positive even when no clinical signs of hepatic involvement were present (104).

This test is a very useful one, a positive result indicating a lesion of the hepatic parenchyma. Unfortunately, it responds more to the acute and advanced types of liver disease, than to the chronic and mild types, and its usefulness is thus, to a certain extent, limited (105,106). Nevertheless, it can be classed among the most reliable tests of hepatic efficiency.

THE STRAUSS TEST.

This was the original method of performing the laevulose test.

100 grams of laevulose are given by the mouth, and the urine collected during the following twenty-four hours.

The sugar, if any, is then estimated in the usual manner. In positive cases, sugar appears in the urine one hour after administration of the laevulose (107).

The results of this test appear to be very indefinite and inconsistent. This appears to be due to the unknown and variable factor of renal permeability. Thus, when 50 g. laevulose were given by the mouth in a series of cases (102) sugar appeared in the urine of some of the normal cases, and did not appear in some of the cases of hepatic disease. This is explained by the fact that the appearance of laevulose in the urine depends more on the kidney threshold for laevulose, than on the liver's efficiency for storing the sugar. Actually, the kidney threshold for laevulose

is lower than for glucose and varies greatly in different individuals.

In a series of cases, laevulose was excreted in the urine, in amounts sufficient to reduce Fehling's solution, by the time the blood-sugar had reached levels varying in different subjects from 0.097% to 0.134%. It would seem, therefore, that in some individuals, a very small amount of laevulose present in the blood will produce glycosuria.

No reliance, therefore, can be placed on this test, and the advantages of estimating the efficiency of the liver by a method which excludes the factor of renal permeability is obvious.

THE GALACTOSE TOLERANCE TEST.

Historical:

The use of galactose as a means of testing liver function was first suggested by Bauer (108) who relied on the urinary findings only for his results. Brasch, (109) showed that galactose is converted by the liver into glycogen, and that its absorption from the alimentary tract takes place at a uniform rate. Roubitschek (110) and Woerner (111) observed galactosuria in animals poisoned with phosphorus. Draudt obtained similar results in animals with an Eck fistula, while Neugebauer (112) demonstrated galactosuria as a constant finding in secondary syphilis. Bauer, in 1924 (113) reaffirmed his reliance on galactosuria as the best test for hepatic function, and even stated that no surgeon should operate till satisfied of the integrity of the liver by means of this test.

In 1919, Kahler and Machold (114) pointed out the renal factor in the galactosuria test, and showed that galactosuria

could occur in the absence of a raised blood sugar. This raised the question of the variable factor of renal permeability, and directed attention to the changes in the blood-sugar level following the ingestion of a given quantity of galactose.

Rowe, in 1924 (115) fixed the kidney threshold for galactose at about 40 gm., stated that the dose is but little if at all influenced by age, weight, or body area, that the chief factor concerned was sex, and suggested the influence of the mammary glands as a possible explanation.

With the advent of micro-methods of blood-sugar estimation, the blood-sugar curve after the administration of given amounts of galactose was then studied, and many continental clinics adopted this method of testing liver efficiency. Davies (116) concluded that the test was of value in the differential diagnosis between obstructive and non-obstructive jaundice. In a comparative study by Elmer and Scheps (117,118), these authors concluded that galactose was superior to laevulose as a liver test. Fischler (119) showed that the metabolism of galactose, unlike that of laevulose, takes place primarily in the liver, though Achard (120) suggested that assimilation by other tissues might be a factor.

In the following pages is given the results of the galactose tolerance test in a series of cases of hepatic and other conditions in which derangement of the carbohydrate metabolic function of the body may occur.

Method:

The galactose used was manufactured by British Drug Houses Limited, and tested for purity by the polarimeter. The average specific rotation was found to be between 79° and 80°, which was above the minimum of 76° prescribed by Bauer.

The test was performed in the morning, fasting, so that a long interval had elapsed after the last meal. Urine was passed just before the test, and examined for abnormal constituents, albumin, sugar, bile, urobilin, etc. 0.2 c.c. of blood was then withdrawn from the ear, and 40 gm. galactose dissolved in 300 c.c. water, swallowed by the patient. The fasting blood-sugar was estimated by McLean's method, which was used throughout. Specimens of blood were taken every half-hour, till the blood-sugar reached its fasting level again, and the results plotted graphically.

The urine was collected at the end of the fourth hour,

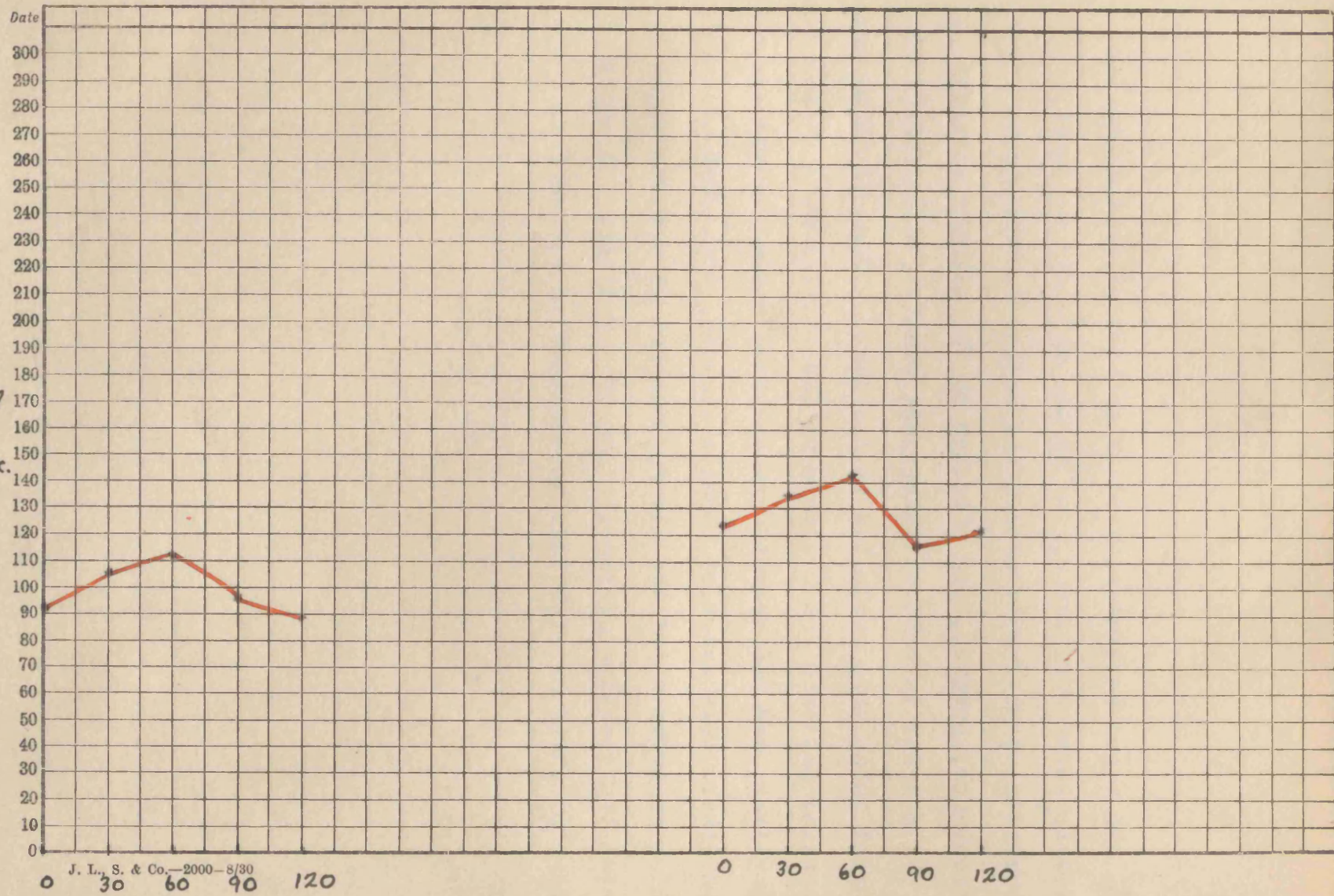
and the sugar estimated; various methods of estimating the urinary sugar were tried; but in the majority of cases, Gerrard's cyano-cupric method was employed. No great importance, however, was attached to the urinary estimations.

In order to control subsequent results, a series of 10 normal cases were subjected to the test. Some of these were hospital patients under treatment for conditions not involving the liver, and the results are included among the normals. The others were cases of hepatic disease, or affections in which enlargement of the liver, jaundice or ascites might raise the question of implication of the liver. A short group of cases of nephritis is included in the series.

CASE 1.
FURUNCULOSIS.

CASE 2.
HEALTHY.

Name.....
Age.....
Ward.....
Journal.....
Page.....



BLOOD SUGAR IN MGM. PER 100 C.C.

Date of Admission.....

J. L. S. & Co.—2000—8/30

TIME IN MINUTES.

Case I.

M.T. Male, 31 yrs. Occupation, Watchman. Complains of recurring boils and abscesses. Urine negative. Diagnosis: Furunculosis.

Fasting	Blood-sugar	0.091%
	30 minutes	0.105
	60 minutes	0.112
	90 minutes	0.096
	120 minutes	0.089
	Maximum Rise	0.021
	Galactosuria	2 gm.

Case 2.

Dr. J.Y. Male, 26 yrs. healthy, urine negative.

Fasting	Blood-sugar	0.124%
	30 minutes.	0.134
	60 minutes.	0.142
	90 minutes.	0.116
	120 minutes.	0.121
	Maximum Rise	0.018
	Galactosuria	1.1 gm.

CASE 3.

HEALTHY.

CASE 4.

DISSEMINATED SCLEROSIS.

Name.....

Age.....

Ward.....

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Date of Admission.....

Case 3.

Dr. E.J. Male, 29 yrs. healthy. Urine negative.

Fasting	Blood-sugar	0.115%
	30 minutes.	0.122
	60 minutes.	0.142
	90 minutes.	0.128
	120 minutes.	0.115
	Maximum Rise	0.027
	Galactosuria	1.5 gm.

Case 4.

H.T. Male. 42 yrs. Engineer; complains of weakness and stiffness of legs, 3 years duration. Spastic gait, knee-jerks exaggerated, positive Babinsky on right side, abdominal reflexes absent, lateral nystagmus, slight intention tremor. Urine negative. Diagnosis, disseminated sclerosis.

Fasting	Blood-sugar	0.127%
	30 minutes.	0.152
	60 minutes.	0.155
	90 minutes.	0.136
	120 minutes	0.129
	Maximum Rise	0.028
	Galactosuria	1.7 gm.

CASE 5.
ACUTE RHEUMATISM.

CASE 6.
HYPERPIESIS.

Name.....
Age.....
Ward.....
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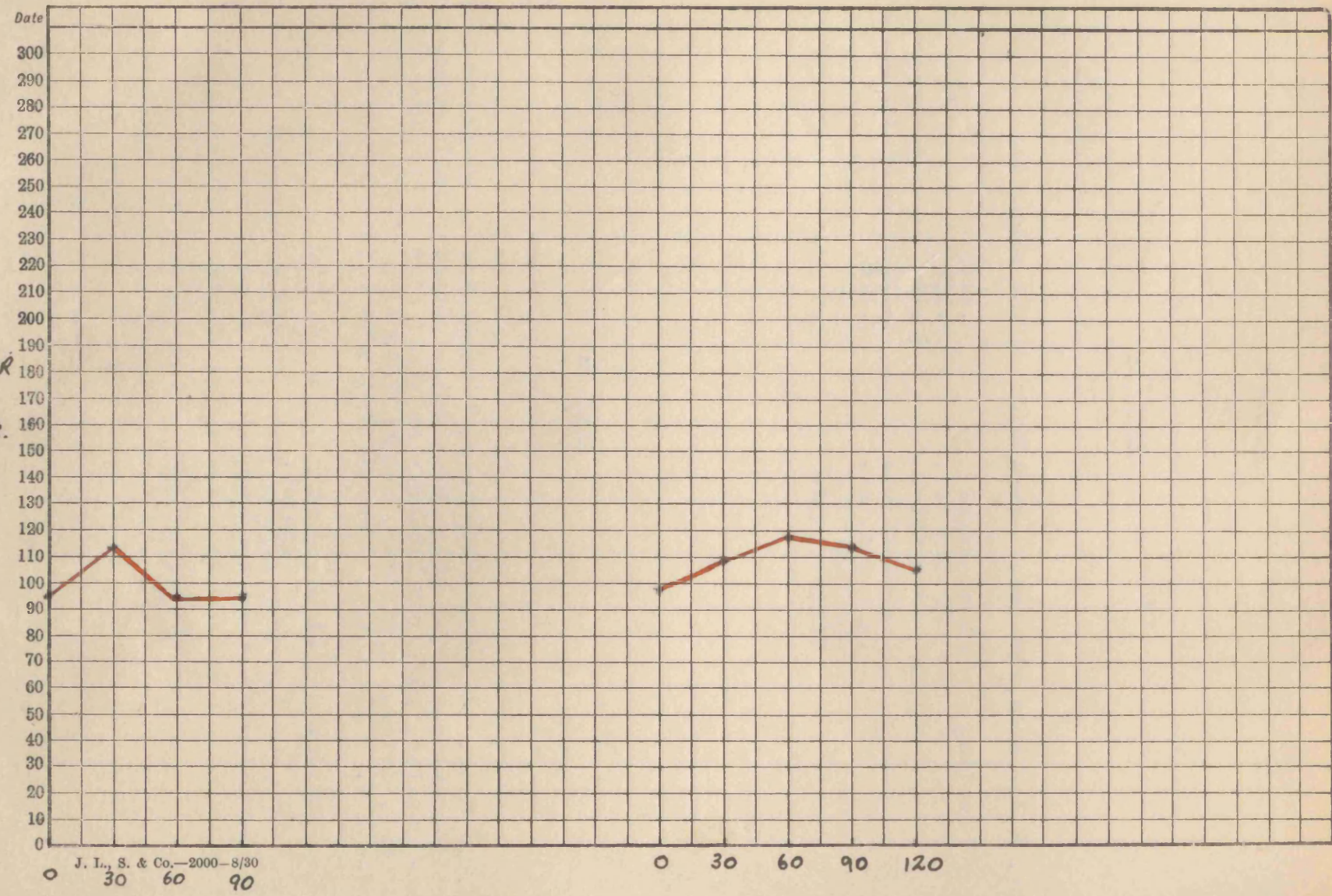

BLOOD SUGAR IN MGM. PER 100 C.C.

Date of Admission.....

J. L., S. & Co.—2000—8/30

TIME IN MINUTES.

Case 5.

T.B. Male, 15 yrs. Admitted 28.1.31 suffering from pains and swelling in joints, 5 days duration. Both ankles, knees, left hip, and right shoulder affected. Presystolic murmur at apex. Urine negative. W.R. negative. Diagnosis, Acute rheumatism.

6.2.31.	Fasting	Blood-sugar	0.095%
		30 minutes.	0.113
		60 minutes.	0.095
		90 minutes.	0.095
		Maximum Rise	0.018
		Galactosuria	a trace.

Case 6.

J.F. Male, 42 yrs. Dairyman, admitted 28.1.31. complaining of giddiness and headaches, 2yrs. duration. Has had several short attacks of unconsciousness. Arteries impalpable, accentuated 2nd aortic sound, B.P. 220/125, W.R. negative, urine negative. Diagnosis, Hyperpiesis.

7.2.31.	Fasting	Blood-sugar	0.098%
		30 minutes.	0.109
		60 minutes.	0.117
		90 minutes.	0.113
		120 minutes.	0.096
		Maximum Rise	0.019
		Galactosuria	0.5 gm.

CASE 7
ANTERIOR POLIOMYELITIS

CASE 8
PARALYSIS AGITANS

Name.....
Age.....
Ward.....
Journal.....
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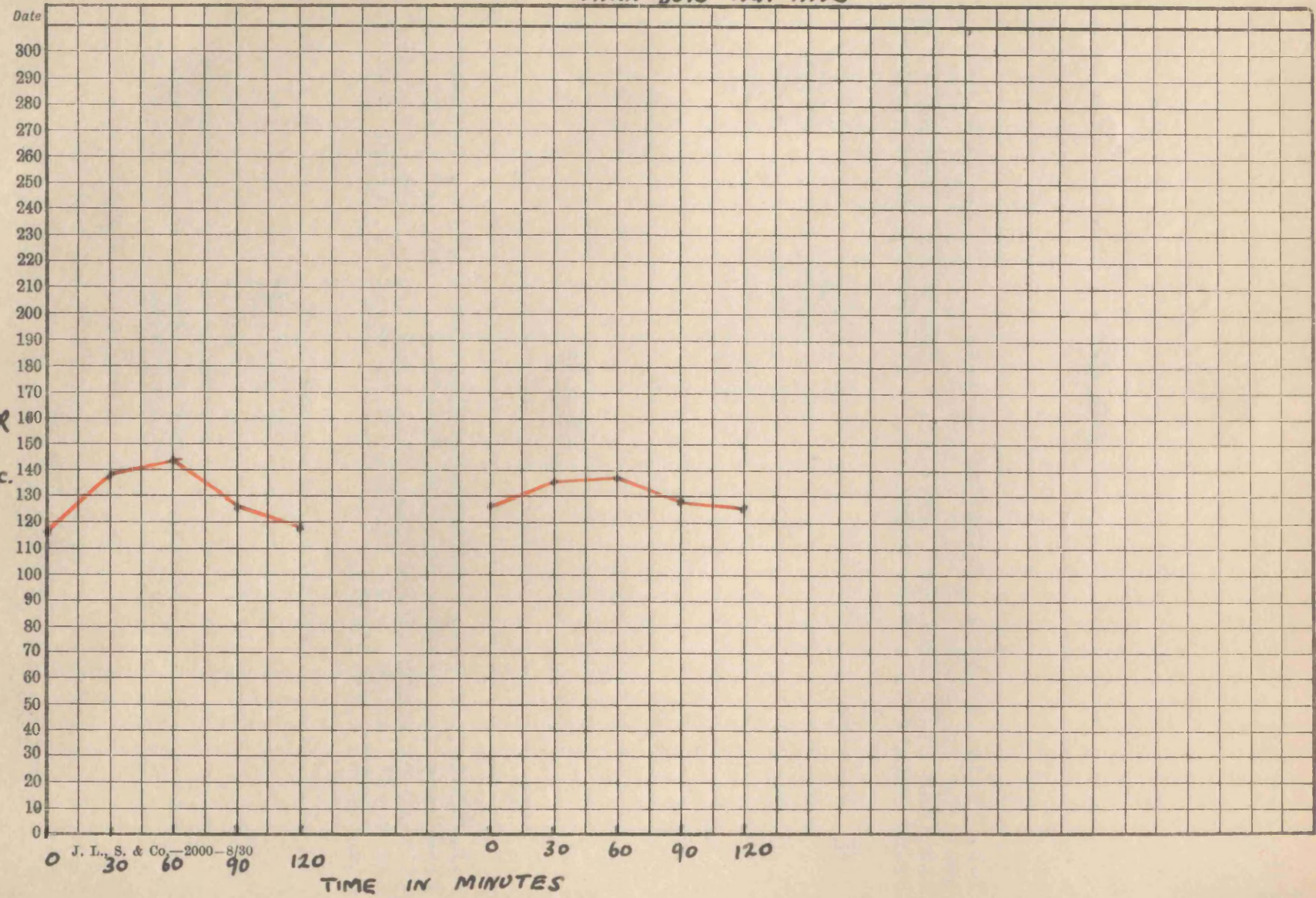

Date of Admission....
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J. L., S. & Co.—2000—8/30

Case 7.

F.H. Male, 24 yrs. Mechanic, admitted 24.12.30. complaining of weakness of legs. Attack of fever 8.9.30. lasting one week. Wasting of muscles of both thighs, with reaction of degeneration. Urine negative. W.R. negative. Diagnosis: Anterior Poliomyelitis.

14.1.31.	Fasting	Blood-sugar	0.117%
		30 minutes	0.139
		60 minutes	0.144
		90 minutes	0.125
		120 minutes	0.117
		Maximum Rise	0.027
		Galactosuria	2.1 gm.

Case 8.

J.C. Male, 57 yrs. Draughtsman, admitted 18.1.31. Weakness and rigidity of arms and legs, tremor of face, tongue and hands, forward sroop of shoulders, retropulsion present, W.R. negative, urine negative. Diagnosis, Paralysis Agitans.

Fasting	Blood-sugar	0.126%
	30 minutes	0.132
	60 minutes	0.136
	90 minutes	0.129
	120 minutes	0.122
	Maximum Rise	0.010
	Galactosuria	0.6 gm.

CASE 9.
HEALTHY.

CASE 10.
HEALTHY.

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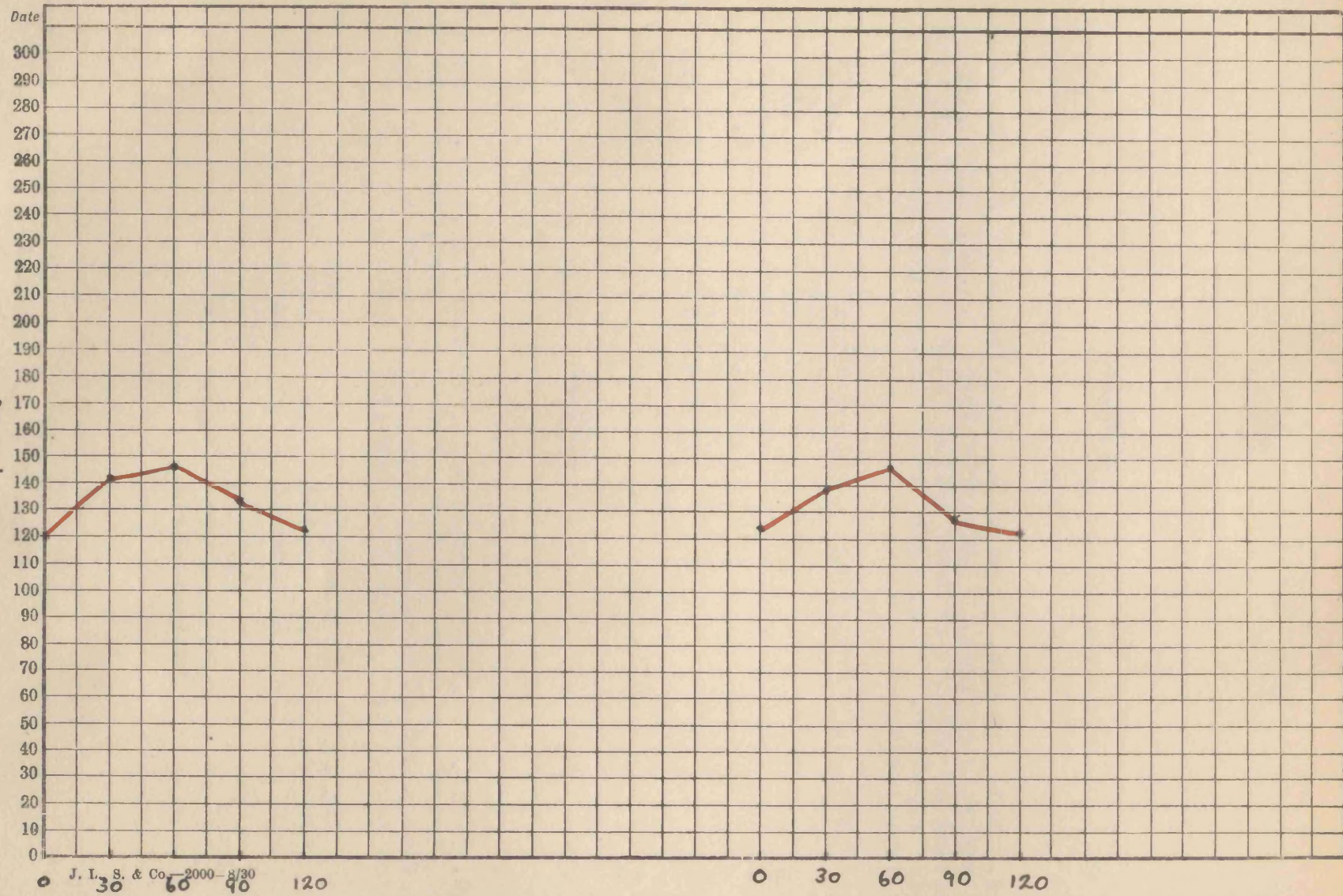

Date of Admission.....
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J. L. S. & Co.—2000—8/30

Case 9.

A.R. 27 yrs. Male, healthy.

Fasting	Blood-sugar	0.120%
	30 minutes	0.141
	60 minutes	0.146
	90 minutes	0.133
	120 minutes	0.122
	Maximum rise	0.026
	Galactosuria	1 gm.

Case 10.

S.M. 32 yrs. Male, healthy.

Fasting	Blood-sugar	0.124%
	30 minutes	0.139
	60 minutes	0.147
	90 minutes	0.128
	120 minutes	0.122
	Maximum Rise	0.023
	Galactosuria	0.7 gm.

CASE 11.

CATARRHAL JAUNDICE.

CASE 12.

CATARRHAL JAUNDICE.

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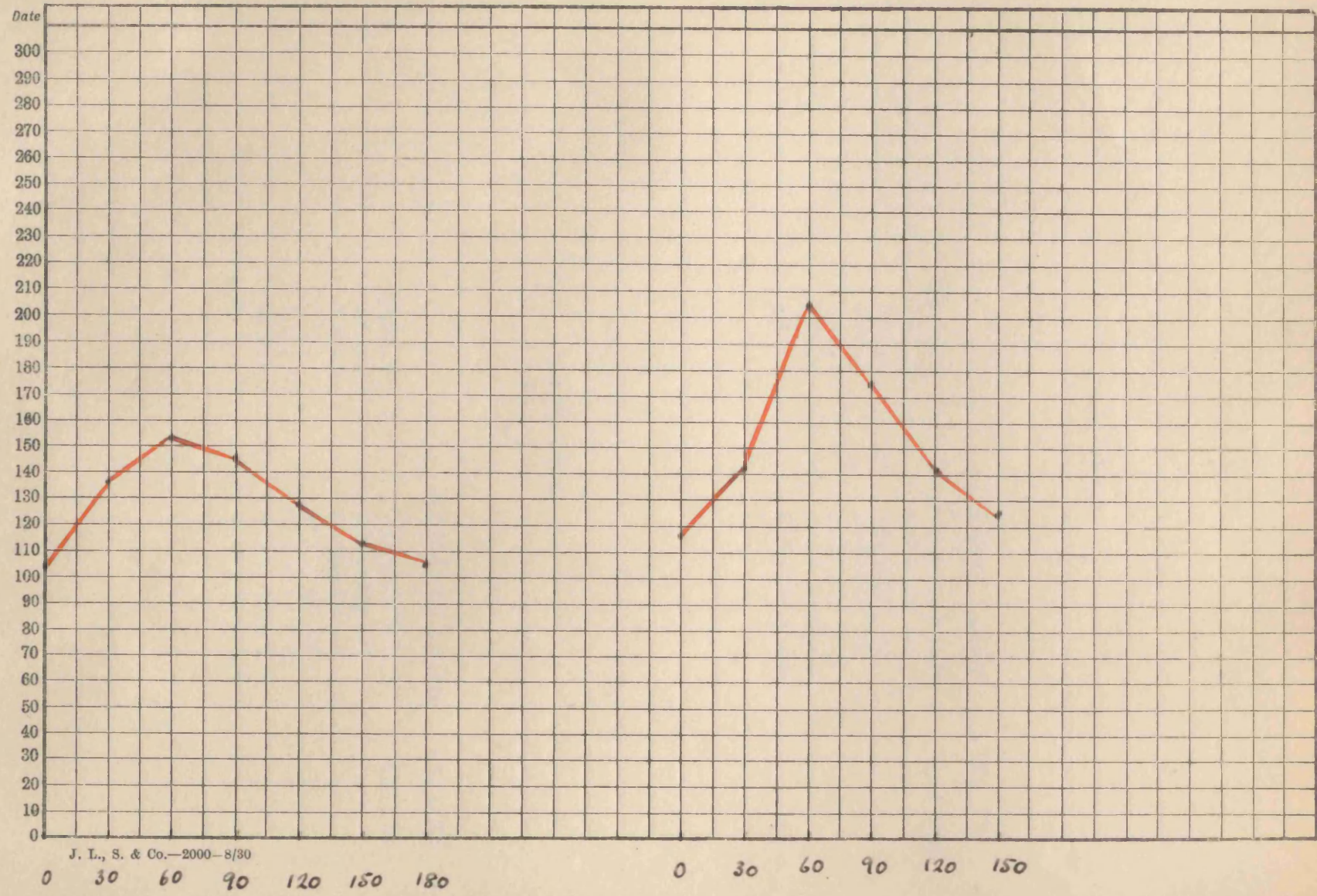

BLOOD SUGAR IN MGM. PER 100 C.C.

Date of Admission.....

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J. L., S. & Co.—2000—8/30

TIME IN MINUTES

Case 11.

D.R. Male, 50 yrs, Meat-porter, jaundice, 4 weeks duration, liver enlarged 1½" below right costal margin, edge and surface smooth. Urine, trace of albumin, and abundant bile present. Jaundice diminishing. Diagnosis, catarrhal jaundice.

Fasting	Blood-sugar	0.106%
	30 minutes	0.137
	60 minutes	0.152
	90 minutes	0.145
	120 minutes	0.128
	150 minutes	0.112
	180 minutes	0.104
	Maximum Rise	0.046
	Galactosuria	2.3 gm.

Case 12.

G.C. Male, 33 yrs. Marine engineer, history of malaria, complaining of jaundice 4 days duration and sickness after meals. No enlargement of liver, abundant bile in urine. Diagnosis catarrhal jaundice.

Fasting	Blood-sugar	0.117%
	30 minutes	0.141
	60 minutes	0.206
	90 minutes	0.175
	120 minutes	0.141
	150 minutes	0.125
	Maximum Rise	0.089
	Galactosuria	4.5 gm.

CASE 13.
CATARRHAL JAUNDICE.

CASE 14.
CATARRHAL JAUNDICE.

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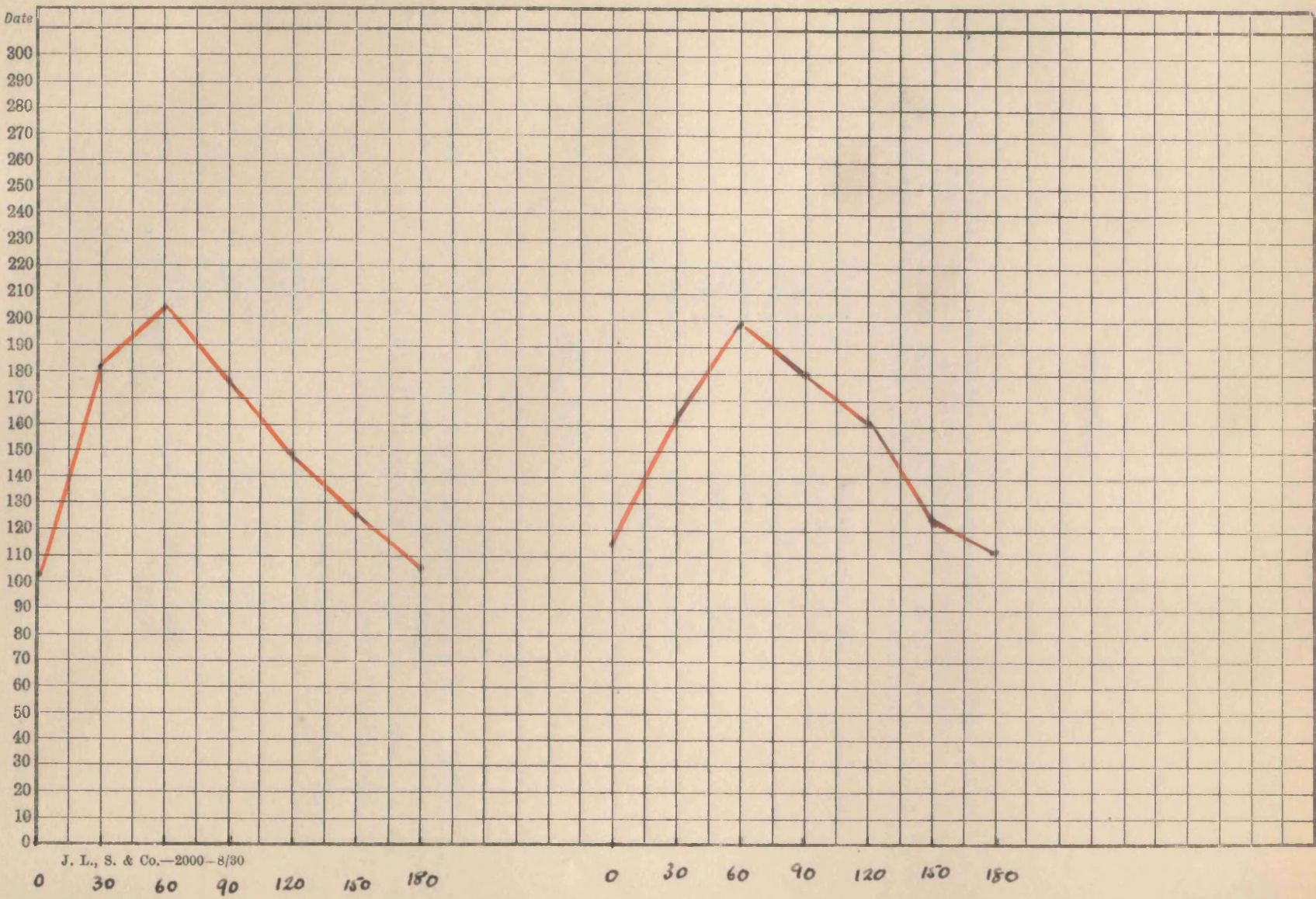

BLOOD SUGAR IN MGM. PER 100 C.C.

Date of Admission.....

J. L., S. & Co.—2000—8/30

TIME IN MINUTES

Case 13.

S.G. Male, 31 yrs. Mechanic, jaundice 11 days duration, slight enlargement of liver, bile and urobilin present in urine. Diagnosis, catarrhal jaundice.

Fasting	Blood-sugar	0.102%
	30 minutes	0.181
	60 minutes	0.204
	90 minutes	0.177
	120 minutes	0.149
	150 minutes	0.126
	180 minutes	0.107
	Maximum rise	0.102
	Galactosuria	3.9 gm.

Case 14.

M.S. Male, 36 yrs. Labourer, sttack of epigastric pain and vomiting, one week duration, followed by jaundice, 5 days duration slight pyrexia, no enlargement of liver, bile present in urine. Diagnosis, catarrhal jaundice.

Fasting	Blood-sugar	0.114%
	30 minutes	0.161
	60 minutes	0.199
	90 minutes	0.180
	120 minutes	0.161
	150 minutes	0.125
	Maximum Rise	0.085
	Galactosuria	3.2 gm.

CASE 15.
CATARRHAL JAUNDICE.

CASE 16.
UNRESOLVED PNEUMONIA.

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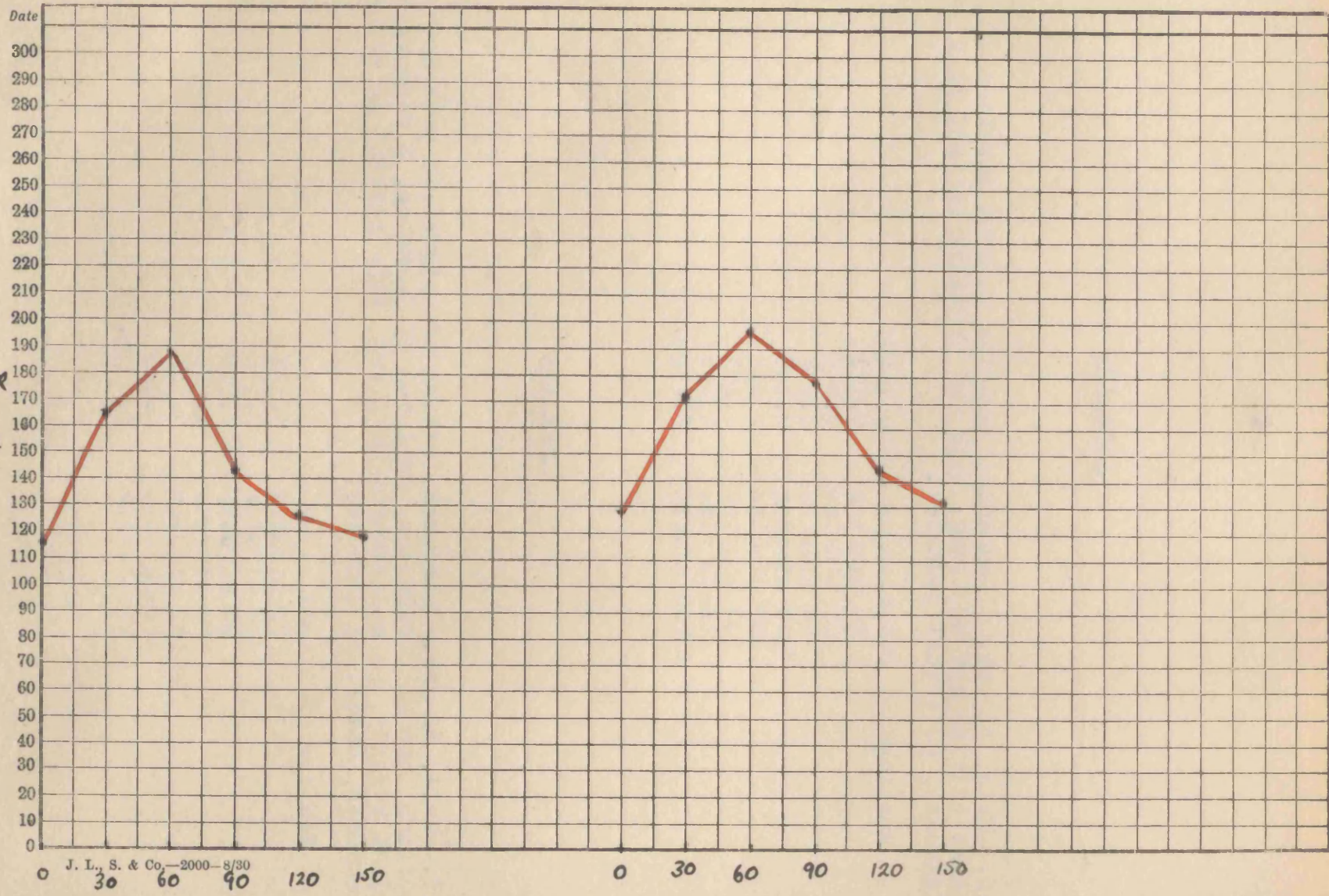

BLOOD SUGAR IN MGM. PER 100 C.C.

Date of Admission.....

J. L., S. & Co.—2000—8/30

TIME IN MINUTES.

Case 15.

N.B. Female, 23 yrs. Weaver, jaundice, 15 days duration, no enlargement of liver, bile present in urine. Diagnosis, catarrhal jaundice.

Fasting	Blood-sugar	0.116%
	30 minutes	0.165
	60 minutes	0.188
	90 minutes	0.142
	120 minutes	0.126
	150 minutes	0.119
	Maximum Rise	0.072
	Galactosuria	2.2. gm.

Case 16.

F.M. Male, 36 yrs. Admitted 4.11.30. suffering from unresolved pneumonia, at right base, 6 weeks duration. On day of test, chest almost clear. Urine negative. Diagnosis: Unresolved Pneumonia.

16.12.30.	Fasting	Blood-sugar	0.128%
		30 minutes	0.172
		60 minutes	0.196
		90 minutes	0.178
		120 minutes	0.144
		150 minutes	0.131
		Maximum Rise	0.068
		Galactosuria	2 gm.

CASE 17.

BRONCHIECTASIS.

CASE 18.

SALVARSAN HEPATITIS

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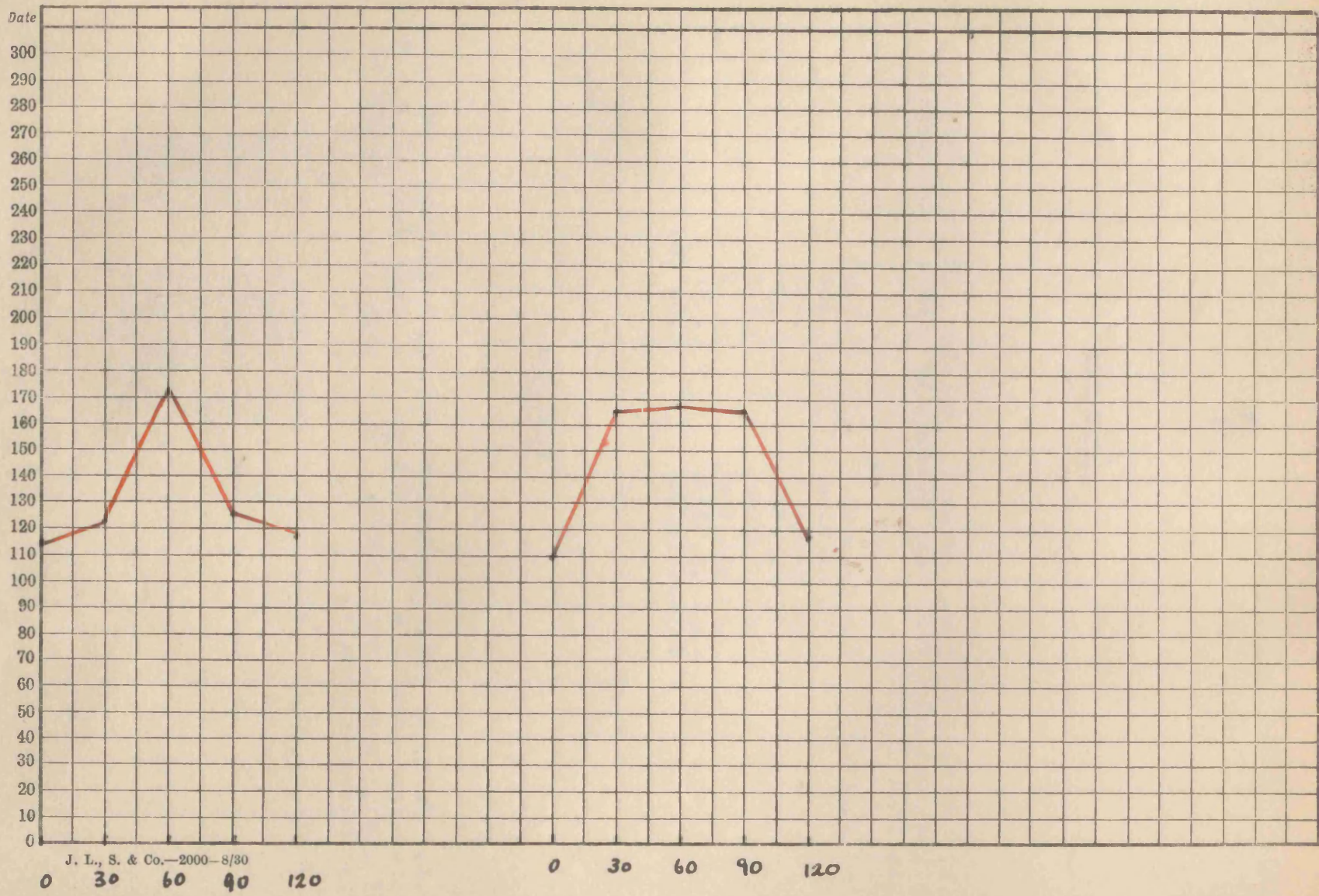

BLOOD SUGAR IN MGM. PER 100 C.C.

Date of Admission.....

J. L., S. & Co.—2000—8/30

TIME IN MINUTES

Case 17.

E.R. Male, 44 yrs. X-ray shows consolidation of right lung with partial pneumothorax, dilatation of bronchi, very abundant sputum, T.B. absent, liver not enlarged, urine negative. Diagnosis: Bronchiectasis.

Fasting	Blood-sugar	0.114%
	30 minutes	0.122
	60 minutes	0.171
	90 minutes	0.125
	120 minutes	0.117
	Maximum Rise	0.057
	Galactosuria	2 gm.

Case 18.

W.C. Male, 47 yrs., tertiary syphilitic aortitis, dilatation of aorta and erosion of vertebrae. Received total of 2.1 gm. kharsivan and 1.2 gm. bismuth, last injection 20.11.30. Jaundice developed 27.11.30, now diminishing. Liver enlarged 2 fingerbreadths below costal margin. Urine, trace of bile. Diagnosis: Salvarsan hepatitis.

23.12.30	Fasting	Blood-sugar	0.110%
		30 minutes	0.165
		60 minutes	0.168
		90 minutes	0.165
		120 minutes	0.118
		Maximum Rise	0.056
		Galactosuria	1.8 gm.

CASE 19

SALVARSAN HEPATITIS

CASE 20.

SALVARSAN HEPATITIS

Name.....

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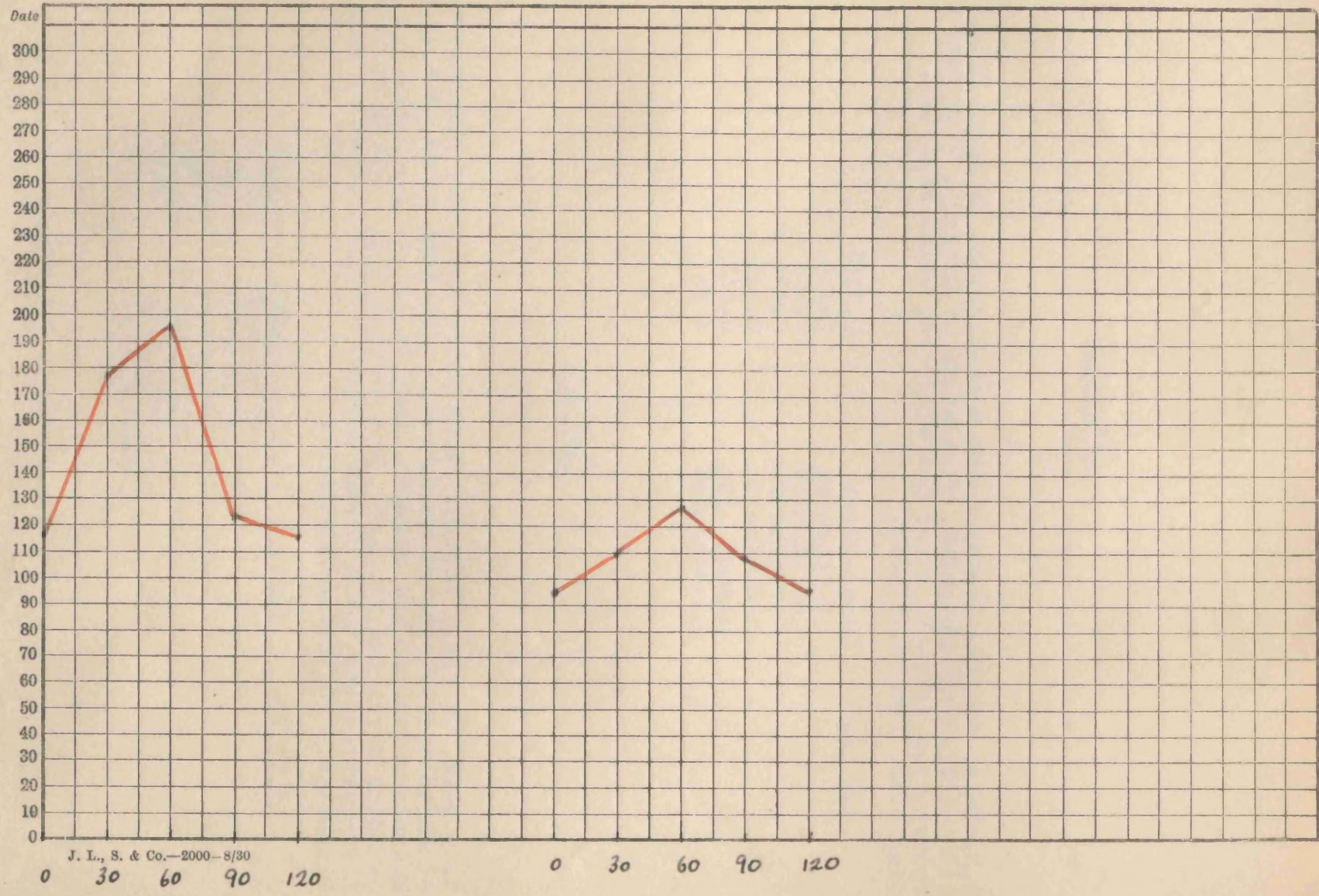

BLOOD SUGAR IN MGM. PER 100 C.C.

Date of Admission.....

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J. L., S. & Co.—2000—8/30

TIME IN MINUTES

Case 19.

A.R. Male, 47 yrs. Secondary syphilitic eruption. Received 4 gm. neokharsivan and 1.6 gm. bismuth till 1.12.30. when jaundice developed. No enlargement of liver, jaundice diminishing, no enlargement of liver. Urine, trace of bile. Diagnosis, salvarsan hepatitis.

24.12.30.	Fasting	Blood-sugar	0.117%
		30 minutes	0.177
		60 minutes	0.196
		90 minutes	0.122
		120 minutes	0.117
		Maximum Rise	0.079
		Galactosuria	2.4 gm.

Case 20.

J.M. Male, 38 yrs., primary syphilis in 1914, gumma of liver in 1922. Received 3 gm. neokharsivan and 1.4 gm. bismuth, developed jaundice on 15.10.30. Jaundice now nearly gone, urine negative. Diagnosis, salvarsan hepatitis.

29.12.30.	Fasting	Blood-sugar	0.095%
		30 minutes	0.110
		60 minutes	0.128
		90 minutes	0.109
		120 minutes	0.095
		Maximum Rise	0.033
		Galactosuria	1 gm.

CASE 21.

SYPHILITIC HEPATITIS.

CASE 22.

SYPHILIS ? ATOPHAN HEPATITIS.

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BLOOD SUGAR IN MGM. PER 100 C.C.

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J. L., S. & Co.—2000—8/30

TIME IN MINUTES.

Case 21.

J.F. Male, 27 yrs. primary syphilis 28.10.30. Received 2.2. gm. neokharsivan and 1.4 gm. bismuth till 19.12.30, when jaundice developed. No enlargement of liver, urine negative, jaundice passing off. Diagnosis, syphilitic hepatitis.

Fasting	Blood-sugar	0.117%
	30 minutes	0.196
	60 minutes	0.264
	90 minutes	0.177
	120 minutes	0.152
	150 minutes	0.137
	180 minutes	0.121
	Maximum Rise	0.147
	Galactosuria	4 gm.

Case 22.

W.M. Male, 56 yrs. Labourer, admitted 30.10.30., complaining of severe headache and occasional diplopia. Auricular fibrillation present. X-ray shows thickening of meninges in occipital region. W.R. positive. Receiving atophan gr. 7½ nocte. Diagnosis. syphilitic pachymeningitis & ? atophan hepatitis.

Fasting	Blood-sugar	0.095%
	30 minutes	0.137
	60 minutes	0.146
	90 minutes	0.121
	120 minutes	0.101
	Maximum Rise	0.051
	Galactosuria	1.6 gm.

CASE 23.

SUBACUTE COMBINED DEGENERATION.

CASE 24.

PERNICIOUS ANAEMIA.

Name.....

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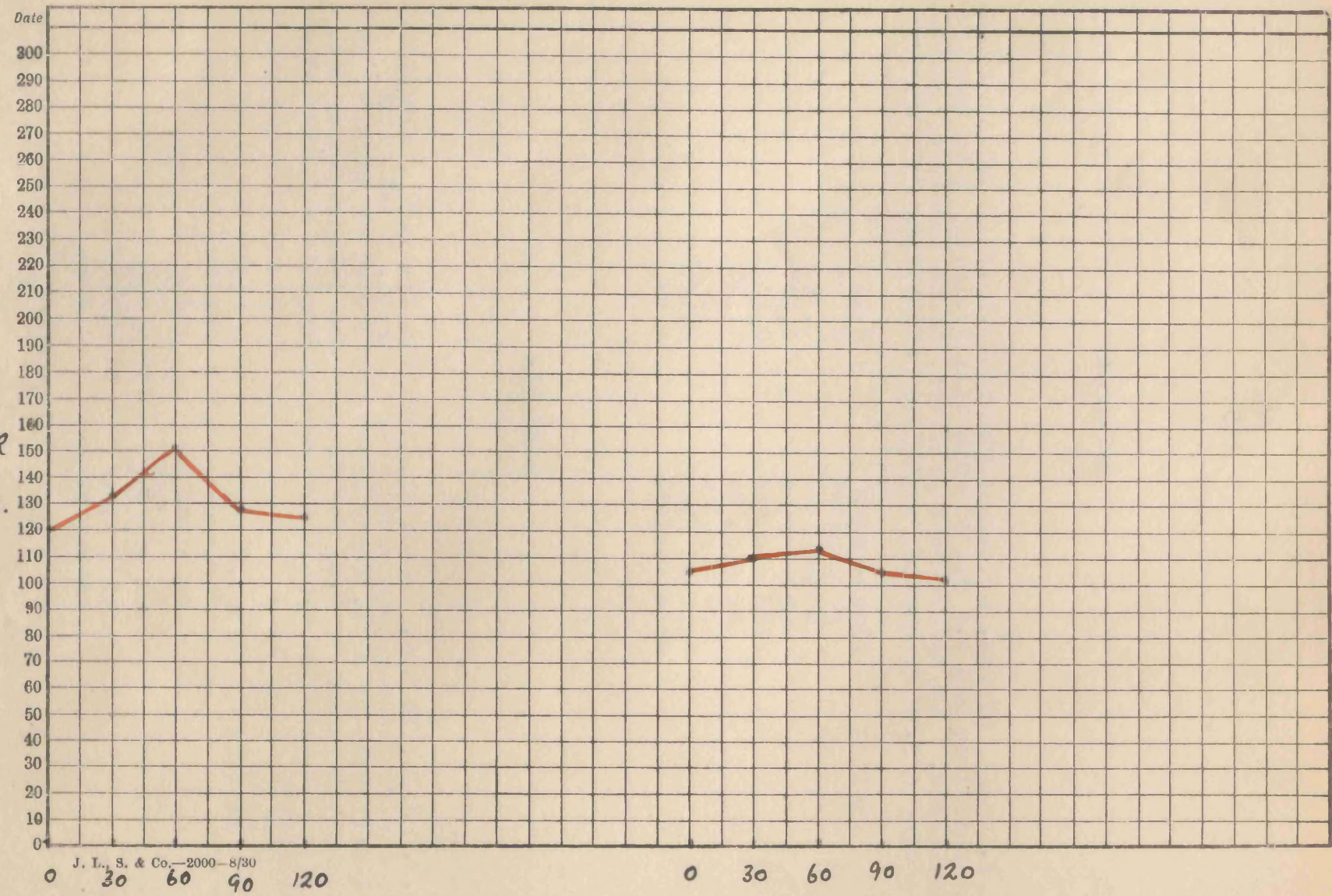

BLOOD SUGAR IN MGM. PER 100 C.C.

Date of Admission.....

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J. L., S. & Co.—2000—8/30

TIME IN MINUTES.

Case 23.

M.S. Female, 49 yrs. Paraesthesia of legs, Babinski positive on both sides, knee-jerk exaggerated on right side, leg muscles flaccid, sensation normal, incoordination of left leg, occasional diplopia, urine negative, achlorhydra present. Red corpuscles 2,400,000, white corpuscles 7,900, Hb 63%. Megalocytes present. Diagnosis subacute combined degeneration.

Fasting	Blood-sugar	0.120%
	30 minutes	0.133
	60 minutes	0.151
	90 minutes	0.128
	120 minutes	0.124
	Maximum Rise	0.031
	Galactosuria	1.2 gm.

Case 24.

M.M. Female, 36 yrs. Admitted 3.1.31 complaining of weakness of legs and swelling of feet. W.R. negative. Red corpuscles 3,900,000, white corpuscles 4,000, Hb 100%, colour index 1.2, megalocytes present, urobilin in urine. Diagnosis: pernicious anaemia.

Fasting	Blood-sugar	0.104%
	30 minutes	0.110
	60 minutes	0.113
	90 minutes	0.107
	120 minutes	0.101
	Maximum Rise	0.009
	Galactosuria	a trace.

CASE 25.
LYMPHATIC LEUKAEMIA

CASE 26
CHOLECYSTITIS

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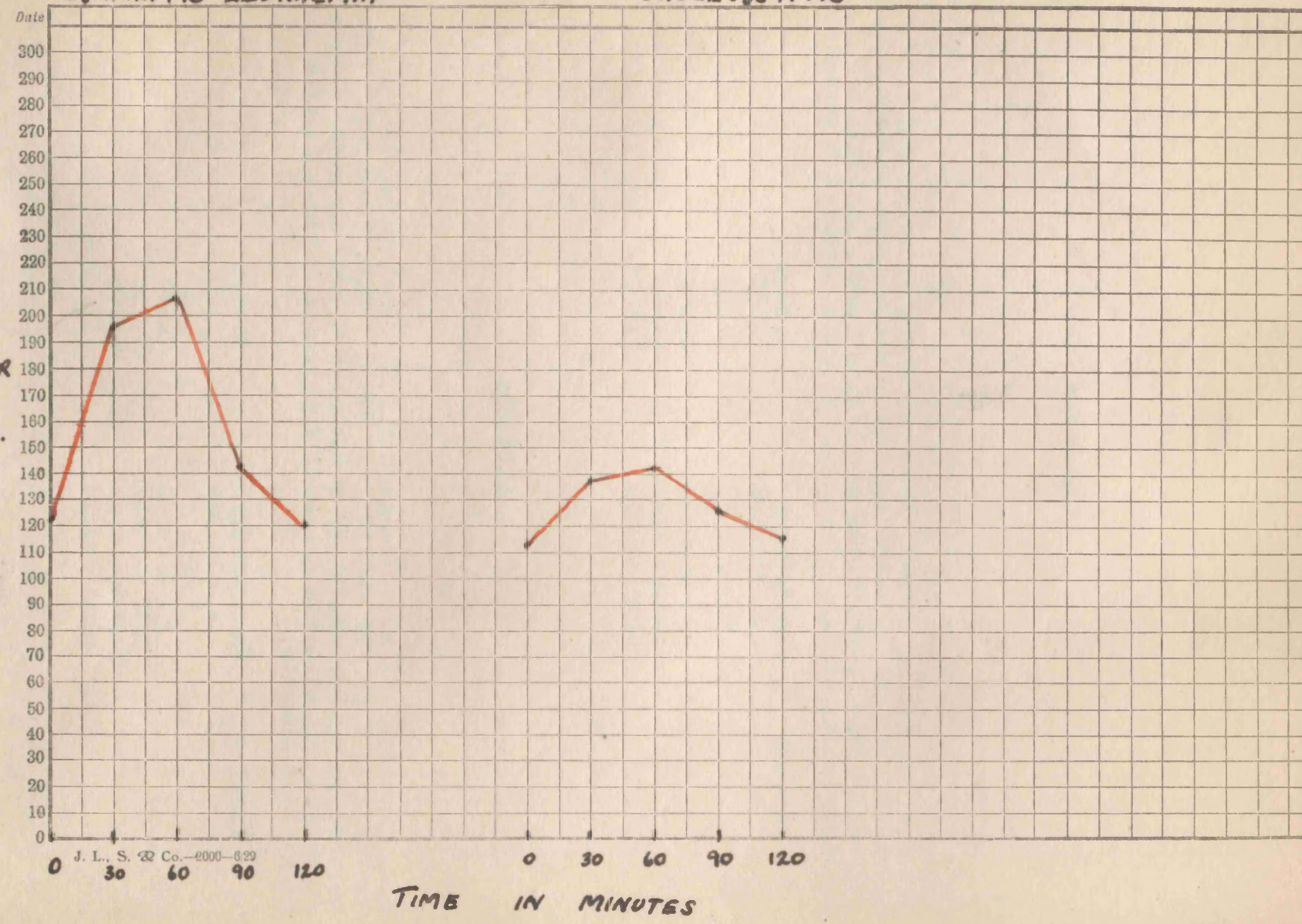

Date of Admission.....
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J. L., S. & Co.—2000—6.29

Case 25.

J.M. Female, 51 yrs, admitted 15.1.31. complaining of weakness and swelling of abdomen, Spleen greatly enlarged extending into right iliac fossa. Red corpuscles 4,300,000, white corpuscles 23,000, Hb 100%,colour index 1.2,lymphocytes 96%. Polymorphs, 3.5%. Urine negative. Diagnosis lymphatic leukaemia.

Fasting	Blood-sugar	0.122%
	30 minutes	0.196
	60 minutes	0.207
	90 minutes	0.141
	120 minutes	0.120
	Maximum Rise	0.085
	Galactosuria	2.8 gm.

Case 26.

E.W. Female, 35 yrs. gall-stones removed in 1927 and 1930. Still having attacks of biliary colic and slight transient jaundice. At operation, stones present in hepatic ducts. Tenderness in gall-bladder region. Urine negative. Liver not enlarged. Diagnosis: Cholecystitis and gall-stones.

Fasting	Blood-sugar	0.112%
	30 minutes	0.138
	60 minutes	0.141
	90 minutes	0.126
	120 minutes	0.114
	Maximum Rise	0.029
	Galactosuria	1.9 gm.

CASE 27
CHOLECYSTITIS

CASE 28
CHOLECYSTITIS & RHEMATOID ARTHRITIS

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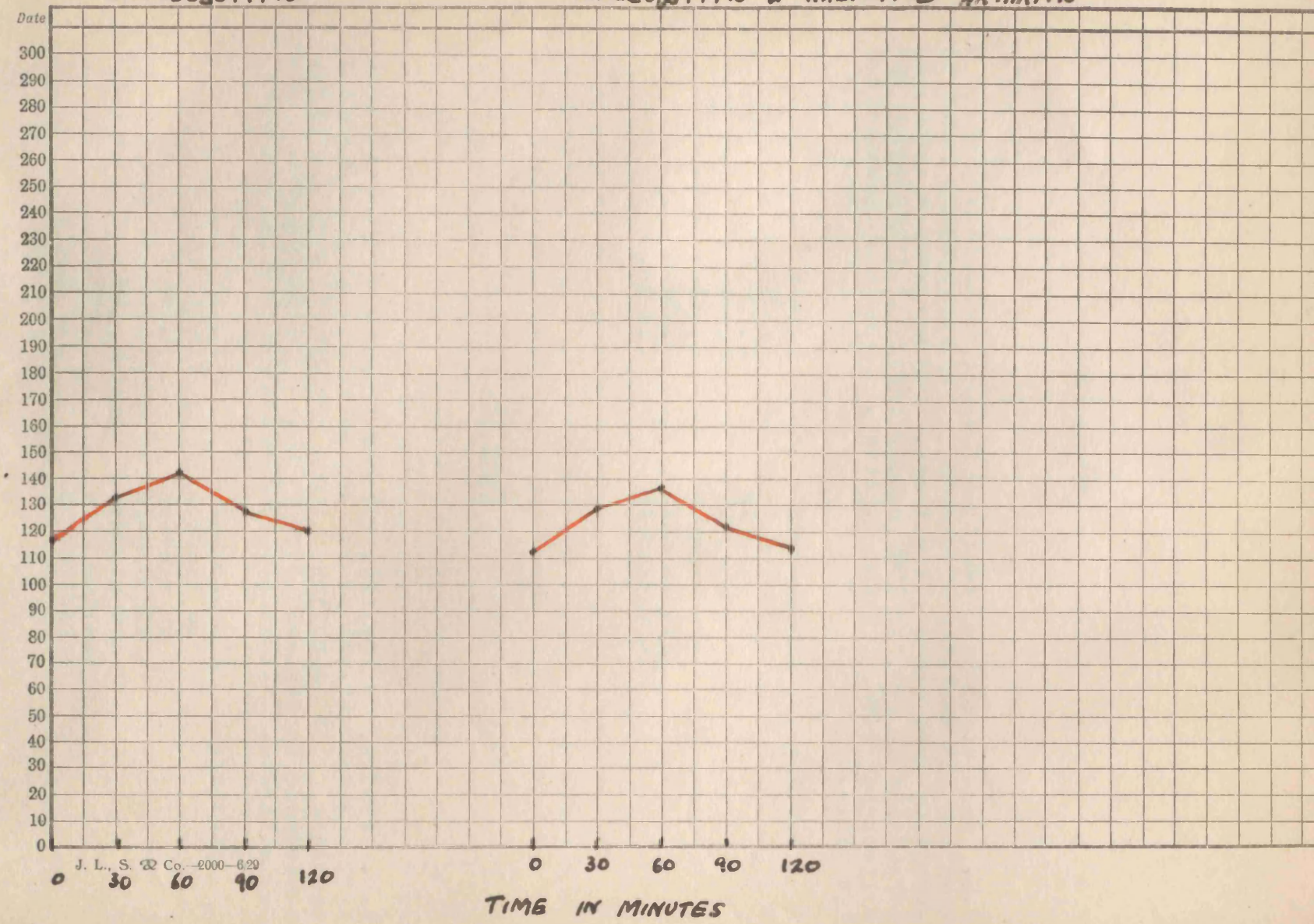

BLOOD SUGAR IN MGM. PER 100 C.C.

Date of Admission.....
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J. L., S. & Co.—2000—6.29

TIME IN MINUTES

Case 27.

J.T. Female, 47 yrs. Admitted 18.1.31. complaining of right hypochondriac pain. Several attacks of biliary colic and jaundice. Marked tenderness in gall bladder region. Liver not enlarged. Urine negative. W.R. negative. Diagnosis: Cholecystitis and gall-stones.

Fasting	Blood-sugar	0.117%
	30 minutes	0.132
	60 minutes	0.142
	90 minutes	0.128
	120 minutes	0.120
	Maximum Rise	0.025
	Galactosuria	0.8 gm.

Case 28.

J.G. Female, 40 yrs. Admitted 23.1.31. complaining of epigastric pain and vomiting 1 hr. after food, and swelling of joints of fingers, elbows, shoulders, ankles, knees and right hip, 2 years duration. Gall-stones removed 5 years ago. Tenderness in gall-bladder region. Urine negative. Diagnosis: Cholecystitis and Rheumatoid arthritis.

Fasting	Blood-sugar	0.112%
	30 minutes	0.129
	60 minutes	0.137
	90 minutes	0.121
	120 minutes	0.112
	Maximum Rise	0.025
	Galactosuria	0.8 gm.

CASE 29
CHOLECYSTITIS

CASE 30
CHOLECYSTITIS

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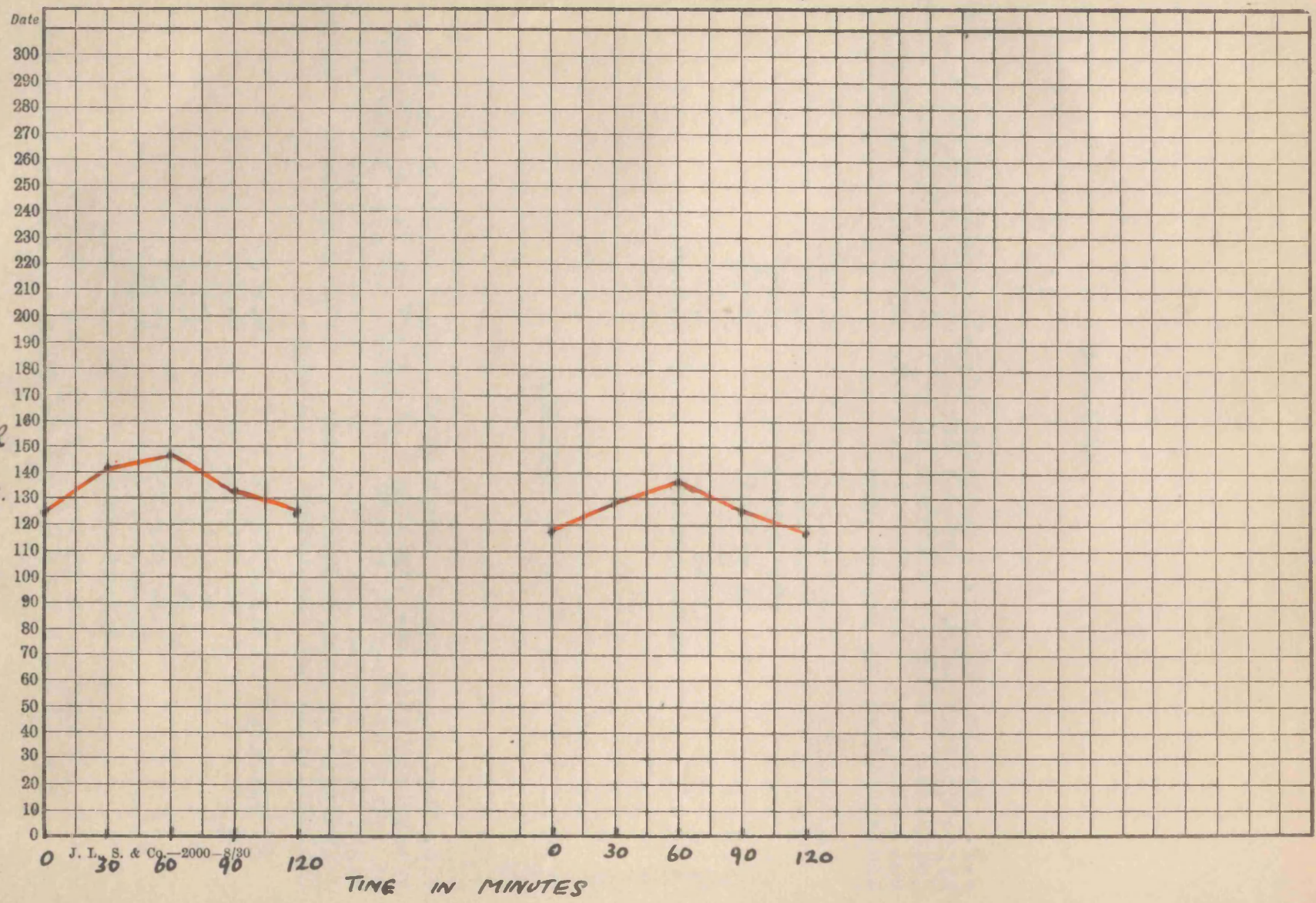

BLOOD SUGAR IN MGM. PER 100 C.C.

Date of Admission.....
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TIME IN MINUTES

Case 29.

S.L. Female, 45 yrs. Several attacks of biliary and ? renal colic. Flatulence after food; marked tenderness and slight rigidity in gall-bladder region. Urine negative. Diagnosis: Cholecystitis and gall-stones.

Fasting	Blood-sugar	0.124%
	30 minutes	0.141
	60 minutes	0.147
	90 minutes	0.131
	120 minutes	0.126
	Maximum Rise	0.023
	Galactosuria	1.2 gm.

Case 30.

M.M. Female, 48 yrs. Attacks of biliary colic and slight jaundice. X-ray shows pyloric adhesions. Pain and vomiting after food. Tenderness in gall-bladder region. Urine negative. Diagnosis: Cholecystitis and gall-stones.

Fasting	Blood-sugar	0.118%
	30 minutes	0.129
	60 minutes	0.137
	90 minutes	0.124
	120 minutes	0.118
	Maximum Rise	0.019
	Galactosuria	a trace.

CASE 31
CHRONIC NEPHRITIS

CASE 32
ACUTE NEPHRITIS

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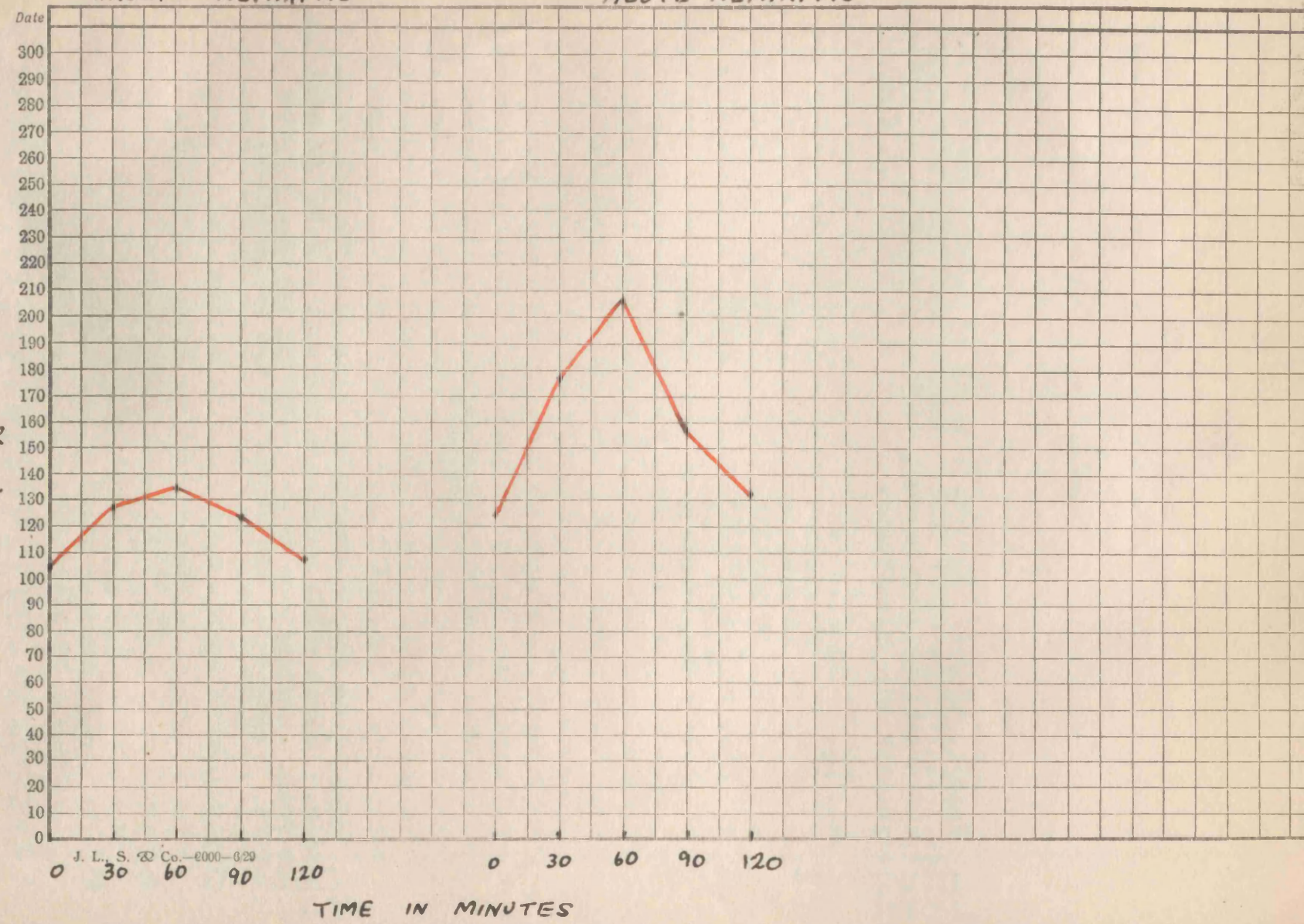


Date of Admission.....

Case 31.

A.L. Male, 55 yrs. Steward, acute nephritis in 1930. Blood pressure 220/130. No oedema. Urine contains large amount of albumin and some casts. Diagnosis: Chronic nephritis.

Fasting	Blood-sugar	0.105%
	30 minutes	0.128
	60 minutes	0.134
	90 minutes	0.123
	120 minutes	0.109
	Maximum Rise	0.029
	Galactosuria	1.2 gm.

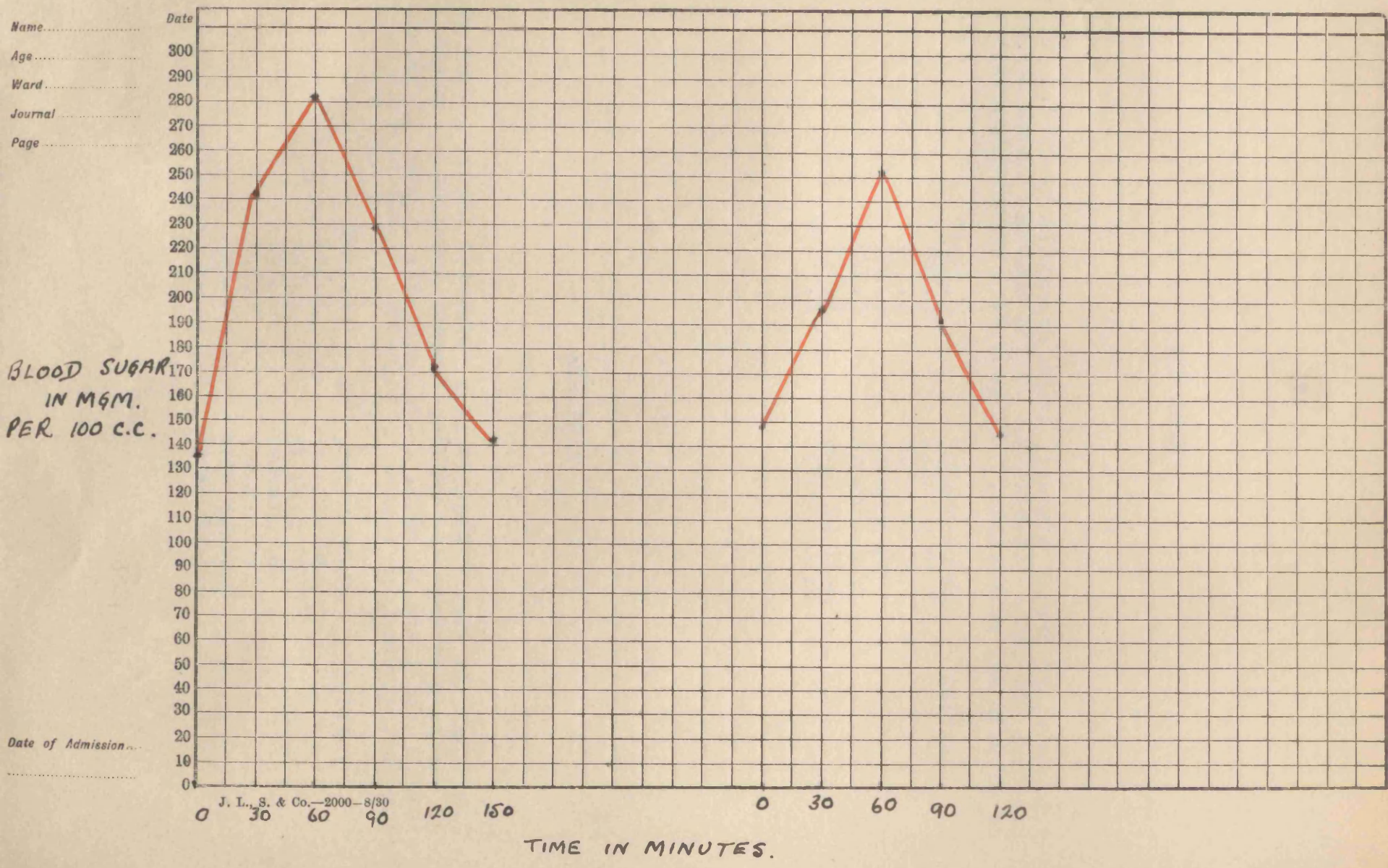
Case 32.

J.B. Male 42 yrs. Steel-worker, admitted 13.1.31. complaining of swelling of feet and ankles, 3 weeks duration. Oedema of thighs, chest and abdomen. W.R. negative. Urine contains abundant albumin, no casts. Diagnosis: Acute nephritis.

Fasting	Blood-sugar	0.124%
	30 minutes	0.177
	60 minutes	0.206
	90 minutes	0.157
	120 minutes	0.131
	Maximum Rise	0.082
	Galactosuria	3.5 gm.

CASE 33.
CHRONIC NEPHRITIS.

CASE 34.
EXOPHTHALMIC GOITRE.



J. L., S. & Co.—2000—8/30

Case 33.

W.M. Male. 33 yrs. Dairyman. Acute nephritis 1915 and 1921. Complains of weakness and headache. W.R. negative. Blood urea 108 mgms%. Non Protein N, 112 mgms%. Urea concentration test, before, 2.1%, 1 hr 1.7%. 2 hrs. 2.1%, 3 hrs, 2.16%. Urine contains albumin and casts. Diagnosis: Chronic nephritis.

Fasting	Blood-sugar	0.135%
	30 minutes	0.241
	60 minutes	0.281
	90 minutes	0.229
	120 minutes	0.171
	150 minutes	0.142
	Maximum Rise	0.146
	Galactosuria	3.8 gm.

Case 34.

J.T. Female, 22 yrs. Maid. Swelling of thyroid 3 years duration, increasing in size during past 4 months. Marked exophthalmos present, pulse 140, systolic murmur at apex, oedema of legs. Urine negative. Diagnosis: Exophthalmic goitre.

Fasting	Blood-sugar	0.148%
	30 minutes	0.196
	60 minutes	0.252
	90 minutes	0.191
	120 minutes	0.145
	Maximum Rise	0.104
	Galactosuria	2.7 gm.

CASE 35.

EXOPHTHALMIC GOITRE.

CASE 36.

T.B. PERITONITIS.

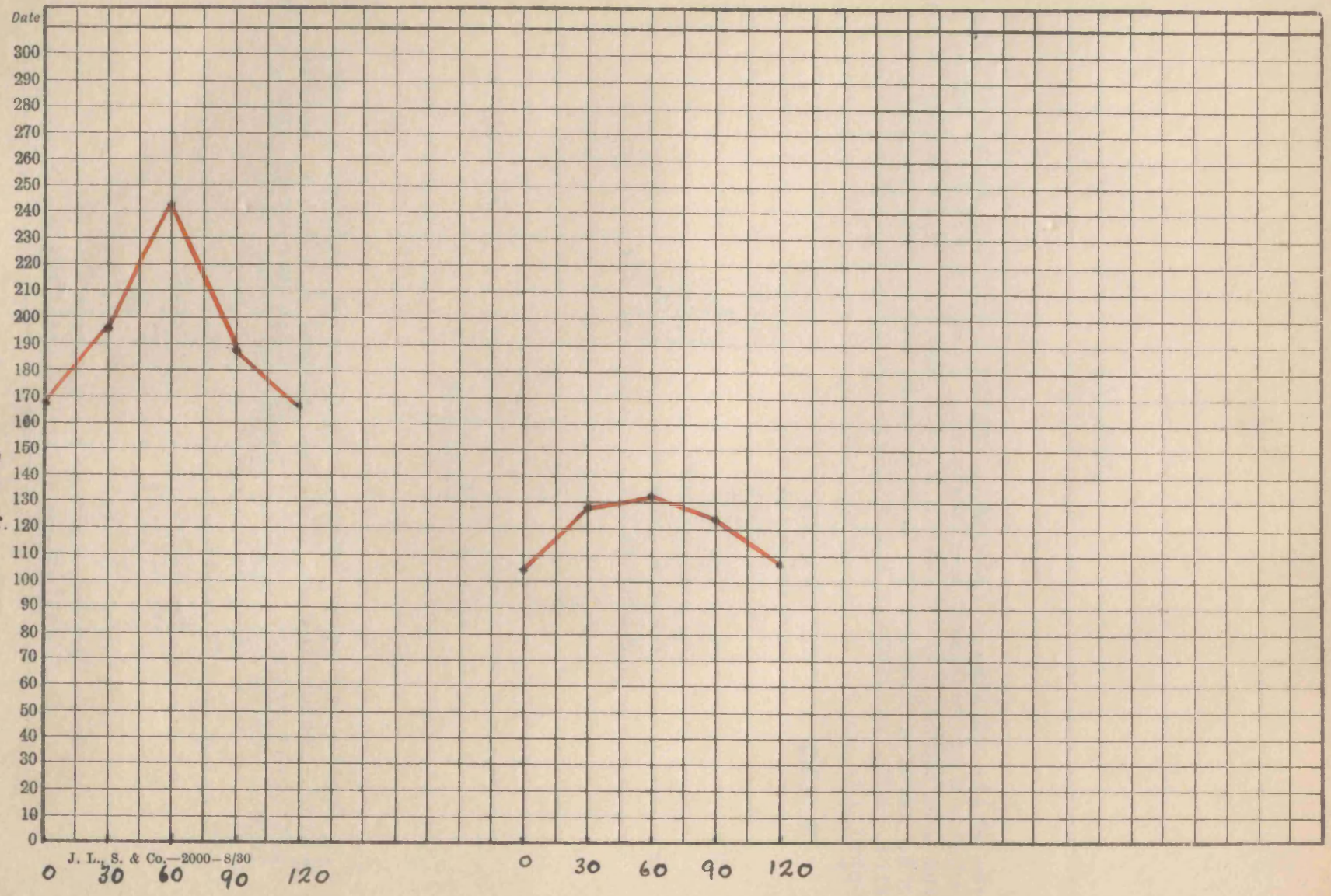
Name.....

Age.....

Ward.....

Journal.....

Page.....



BLOOD SUGAR IN MGM. PER 100 C.C.

Date of Admission.....

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J. L., S. & Co.—2000—8/30

TIME IN MINUTES.

Case 35.

M.S. Female 49 years. Swelling of thyroid 3 years ago, increasing in size during past three months. Marked exophthalmos present. Pulse 150, systolic murmur at apex. Urine contains trace of sugar. Diagnosis: Exophthalmic goitre.

Fasting	Blood-sugar	0.169%
	30 minutes	0.196
	60 minutes	0.241
	90 minutes	0.188
	120 minutes	0.166
	Maximum Rise	0.072
	Galactosuria	4.1 gm.

Case 36.

W.R. Male, 25 yrs. Brick-layer. Admitted 18.1.31 with swelling of abdomen 7 days duration. Marked loss of weight. Large quantity of free fluid present in abdomen. Tapped on 20.1.31. Liver and spleen not enlarged. Doughy masses palpable. Urine negative. Diagnosis: T.B. Peritonitis.

Fasting	Blood-sugar	0.104%
	30 minutes	0.128
	60 minutes	0.131
	90 minutes	0.124
	120 minutes	0.107
	Maximum Rise	0.027
	Galactosuria	1 gm.

CASE 37.

CARCINOMA OF LIVER

CASE 38.

SEC. CARCINOMA OF LIVER

Name.....

Age.....

Ward.....

Journal.....

Page.....

Date

BLOOD SUGAR IN MGM. PER 100 C.C.

300 290 280 270 260 250 240 230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

Date of Admission.....

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0 30 60 90 120

0 30 60 90 120 150 180

TIME IN MINUTES.

Case 37.

J.G. Female, 53 yrs. Rapid loss of weight 8 weeks duration. Liver enlarged to umbilicus, very hard and nodular, edge irregular. No jaundice or ascites. Red corpuscles 2,900,000. White corpuscles 9,000. Hb. 64%, colour index, 1. Urine negative. Diagnosis: Carcinoma of liver.

Fasting	Blood-sugar	0.104%
	30 minutes	0.143
	60 minutes	0.162
	90 minutes	0.146
	120 minutes	0.110
	Maximum Rise	0.042
	Galactosuria	1.2 gm.

Case 38.

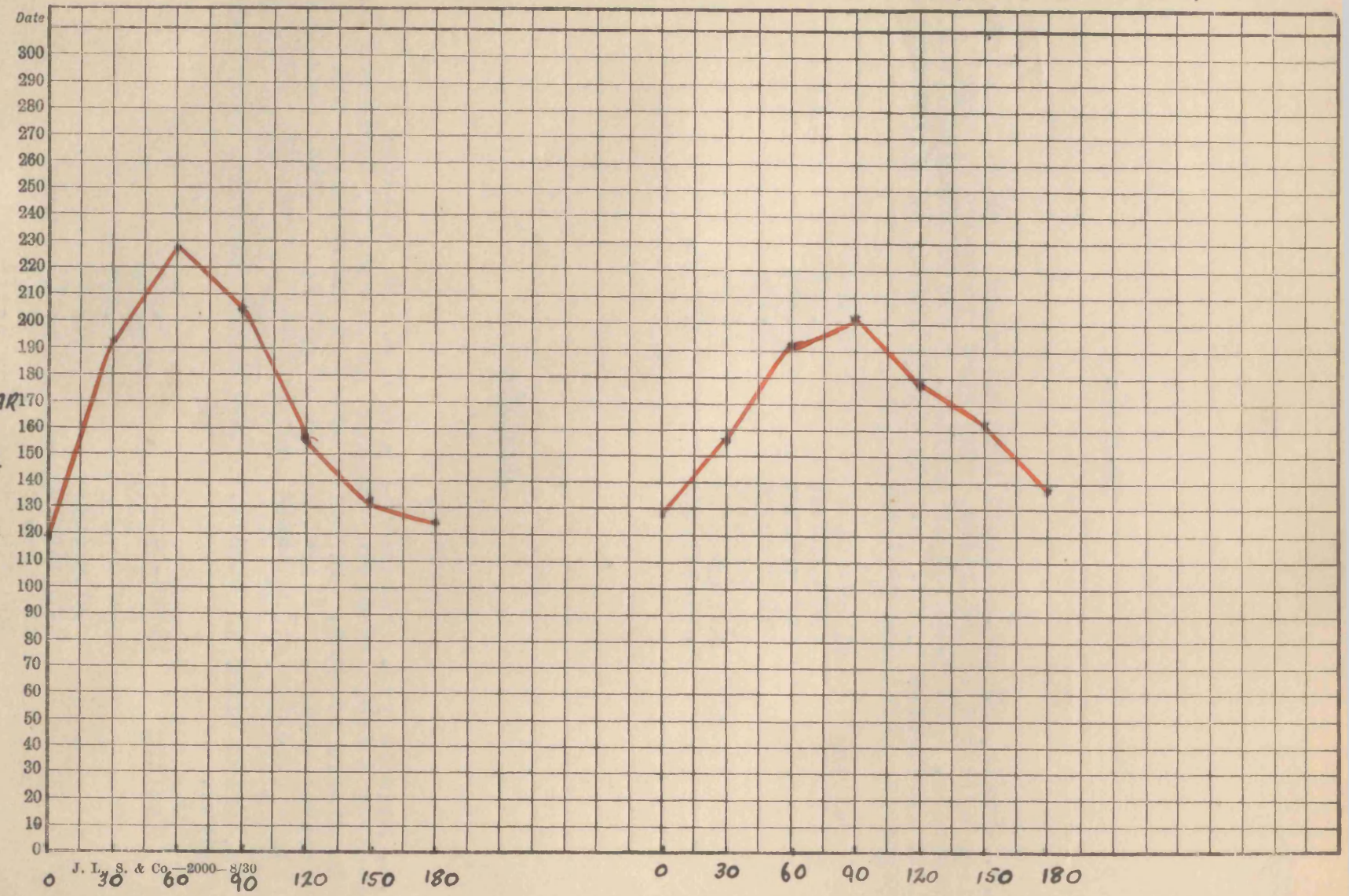
J.W. Male 82 yrs. Abdominal pain 6 months duration, Jaundice 3 weeks duration, varying in intensity, diarrhoea and pale stools present. Purpuric areas in lumbo-sacral and occipital region. Slight oedema of ankles. Liver enlarged 2 fingerbreadths below costal margin, edge irregular, surface hard and nodular masses present in epigastrium. Urine contains bile, fresh blood in stools. Diagnosis: Secondary carcinoma of liver.

Fasting	Blood-sugar	0.166%
	30 minutes	0.187
	60 minutes	0.197
	90 minutes	0.202
	120 minutes	0.202
	150 minutes	0.183
	180 minutes	0.171
	Maximum Rise	0.036
	Galactosuria	1.2 gm.

CASE 39.
CARCINOMA OF LIVER.

CASE 40.
CIRRHOSIS OF LIVER.

Name.....
Age.....
Ward.....
Journal.....
Page.....



BLOOD SUGAR IN MGM. PER 100 C.C.

Date of Admission.....

J. L. S. & Co.—2000—8/30

TIME IN MINUTES.

Case 39.

M.W. Female, 38 yrs. Pains in chest and swelling of abdomen 2 weeks duration. Dulness and absence of breath sounds at right base. Liver enlarged 2 fingerbreadths below costal margin, edge smooth, no nodules present, loss of weight. Urine negative. Diagnosis: ? Carcinoma of liver.

Fasting	Blood-sugar	0.119%
	30 minutes	0.192
	60 minutes	0.227
	90 minutes	0.204
	120 minutes	0.156
	150 minutes	0.133
	180 minutes	0.124
	Maximum Rise	0.085
	Galactosuria	4 gm.

Case 40.

W.W. Male. 63 yrs. Admitted 28.1.30 swelling of abdomen 17 days duration and swelling of legs. Abdomen greatly distended with fluid - on tapping no organisms and a few lymphocytes found. Liver enlarged upwards. W.R. negative. Urine negative. Diagnosis: Cirrhosis of liver.

Fasting	Blood-sugar	0.128%
	30 minutes	0.156
	60 minutes	0.191
	90 minutes	0.202
	120 minutes	0.177
	150 minutes	0.152
	180 minutes	0.137
	Maximum Rise	0.074
	Galactosuria	3 gm.

CASE 41.
CIRRHOSIS OF LIVER.

CASE 42.
CARCINOMA OF PANCREAS.

Name.....
Age.....
Ward.....
Journal.....
Page.....

Date

BLOOD SUGAR IN MGM. PER 100C.C.

300
290
280
270
260
250
240
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

Date of Admission.....

J. L., S. & Co.—2000—6.29

0 30 60 90 120

0 30 60 90 120

TIME IN MINUTES.

Case 41.

M.T. Female, 48 yrs. Swelling of abdomen. 12 months duration. Dilated superficial veins on abdomen. Ascites present, moderate, liver enlarged 2 fingerbreadths below ribs, edge hard and smooth. Urine negative. Diagnosis: Cirrhosis of liver.

Fasting	Blood-sugar	0.141%
	30 minutes	0.156
	60 minutes	0.196
	90 minutes	0.128
	120 minutes	0.136
	Maximum Rise	0.055
	Galactosuria	2.2. gm.

Case 42.

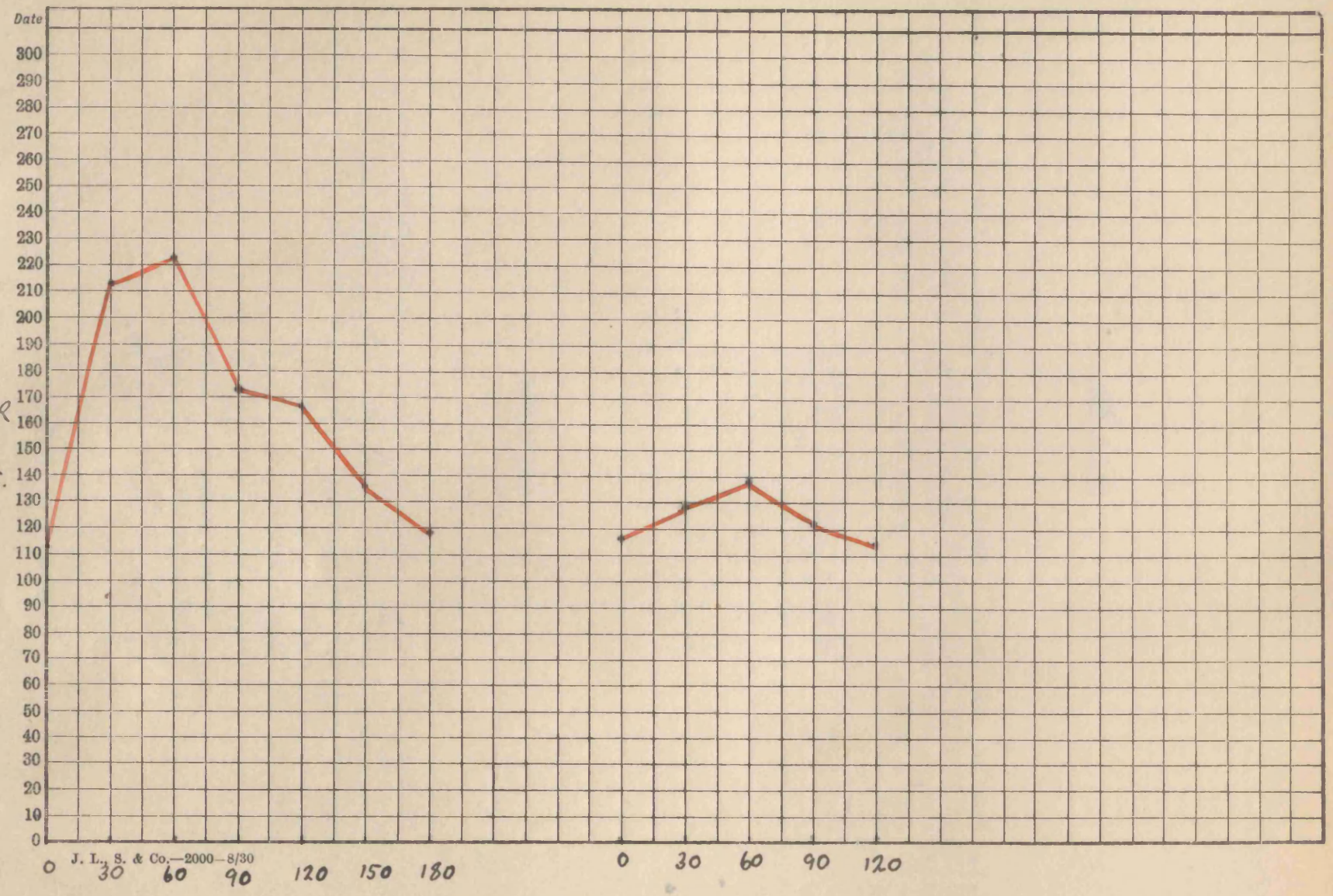
J.L. Female, 43 yrs. Loss of weight and abdominal pain 6 months duration. Jaundice 3 weeks duration. Diarrhoea and pale stools containing no bile pigments. Urine contains bile, diastase 30 units. Diagnosis: Carcinoma of head of pancreas.

Fasting	Blood-sugar	0.097%
	30 minutes	0.118
	60 minutes	0.122
	90 minutes	0.112
	120 minutes	0.094
	Maximum Rise	0.025
	Galactosuria	0.8 gm.

CASE 43.
CIRRHOSIS OF LIVER.

CASE 44.
CARDIAC FAILURE.

Name.....
Age.....
Ward.....
Journal.....
Page.....



Date of Admission.....

J. L., S. & Co.—2000—8/30

Case 43.

M.S. Female 47 yrs. Gastritis many years duration, jaundice 3 weeks duration, loss of weight, caput medusae present, liver just palpable, edge hard, no ascites. Urine: bile present. Diagnosis: Cirrhosis of liver.

Fasting	Blood-sugar	0.112%
	30 minutes	0.213
	60 minutes	0.222
	90 minutes	0.172
	120 minutes	0.166
	150 minutes	0.137
	180 minutes	0.118
	Maximum Rise	0.110
	Galactosuria	3.2 gm.

Case 44.

O.B. Male, 52 years. Chronic bronchitis, long duration, heart enlarged, systolic murmur at apex, pain in hypochondrium, liver enlarged 1" below costal margin, smooth hard edge, oedema of feet, marked dyspnoea. Urine negative. Diagnosis: Cardiac Failure.

Fasting	Blood-sugar	0.116%
	30 minutes	0.129
	60 minutes	0.138
	90 minutes	0.121
	120 minutes	0.116
	Maximum Rise	0.022
	Galactosuria	1 gm.

CASE 45.

CARDIAC FAILURE.

Name.....

Age.....

Ward.....

Journal.....

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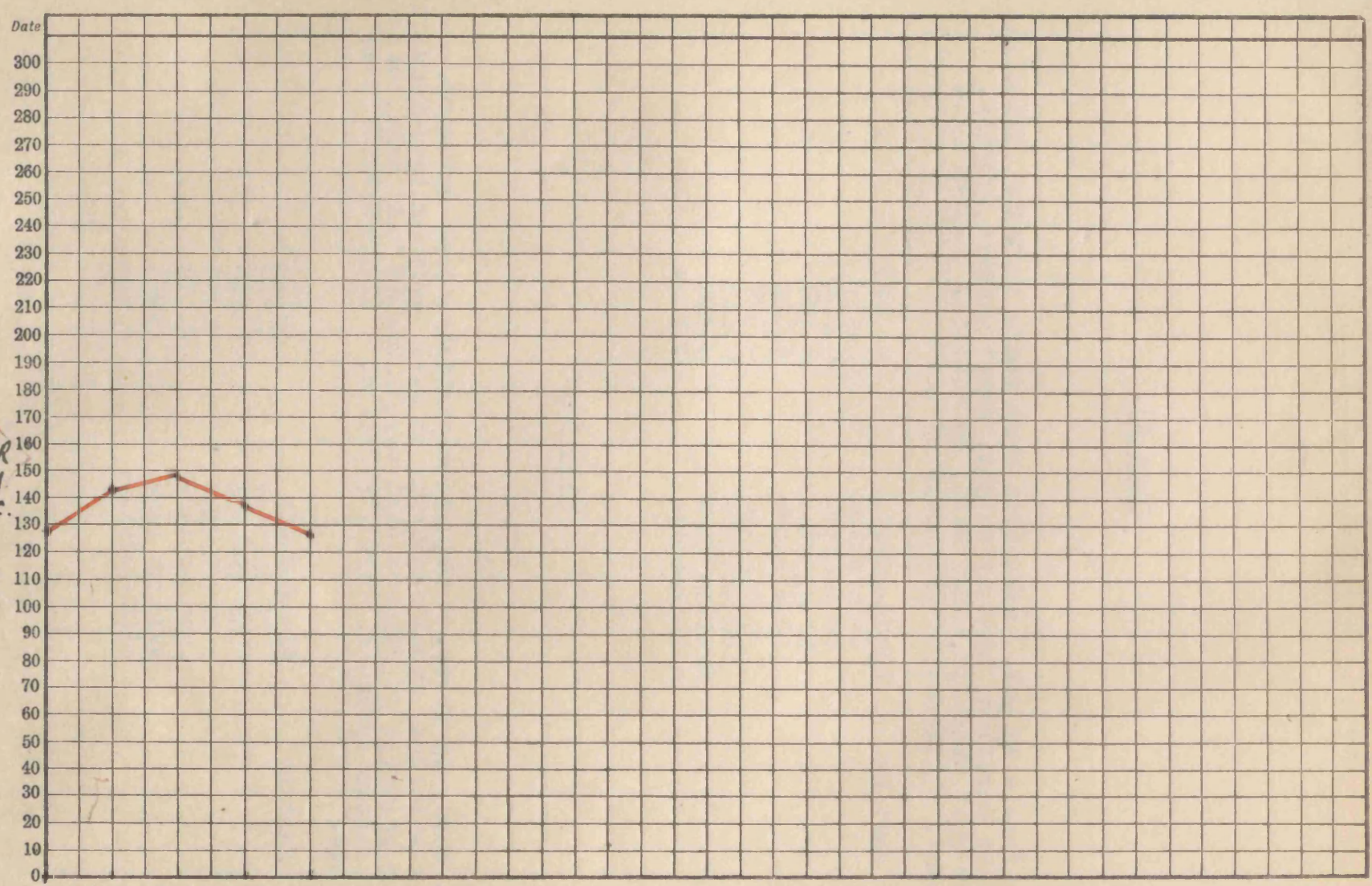

Date of Admission.....

Case 45.

S.M. Male, 42 yrs. Gassed during war, chronic bronchitis, dyspnoea, heart dilated, yellow conjunctivae, liver palpable, edge smooth, oedema of ankles. Urine negative. Diagnosis: Cardiac failure.

Fasting	Blood-sugar	0.128%
	30 minutes	0.141
	60 minutes	0.149
	90 minutes	0.137
	120 minutes	0.125
	Maximum Rise	0.021
	Galactosuria	0.5 gm.

TABULATED RESULTS OF CASES.

No.	Sex	Age	DIAGNOSIS	BLOOD SUGAR %						Galactosuria in gm.
				Fasting	30 Mins.	60 Mins.	90 Mins.	120 Mins.	Rise in Mgm%	
1	M	31	Furunculosis	0.091	0.105	0.112	0.096	0.089	21	2
2	M	26	Healthy	0.124	0.134	0.142	0.116	0.121	18	1.1
3	M	29	Healthy	0.115	0.122	0.142	0.128	0.115	27	1.5
4	M	42	Dissem.Sclerosis	0.127	0.152	0.155	0.136	0.129	28	1.7
5	M	15	Acute Rheumatism	0.095	0.113	0.095	0.095	0.095	18	Trace
6	M	42	Hyperpiesis	0.098	0.109	0.117	0.113	0.096	19	0.5
7	M	24	Ant.Poliomyelitis	0.117	0.139	0.144	0.125	0.117	27	2.1
8	M	57	Paral. Agitans	0.126	0.132	0.136	0.129	0.122	10	0.6
9	M	27	Healthy	0.120	0.141	0.146	0.133	0.122	26	1
10.	M	32	Healthy	0.124	0.139	0.147	0.128	0.122	23	0.7
11	M	50	Catar. Jaundice	0.106	0.137	0.152	0.145	0.128	46	2.3
12	M	33	Catar. Jaundice	0.117	0.141	0.206	0.175	0.141	89	4.5
13	M	31	Catar. Jaundice	0.102	0.181	0.204	0.177	0.149	102	3.9
14	M	36	Catar. Jaundice	0.114	0.161	0.199	0.180	0.161	85	3.2
15	F	23	Catar. Jaundice	0.116	0.165	0.188	0.142	0.126	72	2.2
16	M	36	Unres. Pneumonia	0.128	0.172	0.196	0.178	0.144	68	2
17	M	44	Bronchiectasis	0.114	0.122	0.171	0.125	0.117	57	2
18	M	47	Salvar. Hepatitis	0.110	0.165	0.168	0.165	0.118	56	1.8
19	M	47	Salvar. Hepatitis	0.117	0.177	0.196	0.122	0.117	79	2.4
20	M	38	Salvar. Hepatitis	0.095	0.110	0.128	0.109	0.095	33	1
21	M	27	Syphil. Hepatitis	0.117	0.196	0.264	0.177	0.152	147	4
22	M	56	Syphil.?Atophan	0.095	0.137	0.146	0.121	0.101	51	1.6
23	F	49	Subac.Comb.Degen	0.120	0.133	0.151	0.128	0.124	31	1.2
24	F	36	Pern.Anaemia	0.104	0.110	0.113	0.107	0.101	9	Trace
25	F	61	Lymph. Leuk.	0.122	0.196	0.207	0.141	0.120	85	2.8

No.	Sex	Age	DIAGNOSIS	BLOOD SUGAR %						Galactosuria in gm.
				Fasting	30 Mins.	60 Mins.	90 Mins.	120 Mins.	Rise in Mgm.	
26	F	35	Cholecystitis	0.112	0.138	0.141	0.126	0.114	29	1.9
27	F	47	Cholecystitis	0.117	0.132	0.142	0.128	0.120	25	0.8
28	F	40	Cholecystitis	0.112	0.129	0.137	0.121	0.112	25	0.8
29	F	45	Cholecystitis	0.124	0.141	0.147	0.131	0.126	23	1.2
30	F	48	Cholecystitis	0.118	0.129	0.137	0.124	0.118	19	Trace
31	M	55	Chron.Nephritis	0.105	0.128	0.134	0.123	0.109	29	1.2
32	M	42	Acute Nephritis	0.124	0.177	0.206	0.157	0.131	82	3.5
33	M	33	Chron.Nephritis	0.135	0.241	0.281	0.229	0.171	146	3.8
34	F	22	Exoph. Goitre	0.148	0.196	0.252	0.191	0.145	104	2.7
35	F	49	Exoph. Goitre	0.169	0.196	0.241	0.188	0.166	72	4.1
36	M	25	T.B.Peritonitis	0.104	0.128	0.131	0.124	0.107	27	1
37	F	53	Carc. of Liver	0.104	0.143	0.162	0.146	0.110	42	1.2
38	M	82	Sec.Carc.of Liver	0.166	0.187	0.197	0.202	0.202	36	1.2
39	F	38	Carc. of Liver	0.119	0.192	0.227	0.204	0.156	85	4
40	M	63	Cirr. of Liver	0.128	0.156	0.191	0.202	0.177	74	3
41	F	48	Cirr. of Liver	0.141	0.156	0.196	0.128	0.136	55	2.2
42	F	43	Carc. of Pancreas	0.097	0.118	0.122	0.112	0.094	25	0.8
43	F	47	Cirr. of Liver	0.112	0.213	0.222	0.172	0.166	110	3.2
44	M	52	Cardiac Failure	0.116	0.129	0.138	0.121	0.116	22	1
45	M	42	Cardiac Failure	0.128	0.141	0.149	0.137	0.125	21	0.5

Analysis of Results:

1. Normal cases: of the 10 control cases, the smallest increase is 10 mgm%, and the highest 28. The average rise is 21.7.

In some cases the maximum increase occurs in half an hour, as in case 5, the blood-sugar returning to normal in one hour; this is exceptional, and probably indicates a liver whose galactose-storing mechanism is very sensitive. This may be an inherited characteristic. More usually, the maximum is reached in one hour, and another hour elapses before the fasting level is regained. Not infrequently, a slight hypoglycaemia occurs at the end of this period, the blood-sugar at the end of two hours being slightly lower than the original fasting-level.

It would seem that the concentration of galactose in the blood requires to reach a certain level before the liver is stimulated to commence the conversion of galactose into glycogen. Once the process is started however, it apparently "overshoots the mark", with a resulting hypoglycaemia.

The maximum rise in the blood-sugar compatible with the

assumption of normal carbohydrate metabolism following the ingestion of 40 gm. of galactose may be taken as being in the region of 30 mgm. Figures over 30 are abnormal. If a sufficiently large number of normal individuals were examined, it would probably be found that a small proportion of them possessed a smaller tolerance for galactose than the others, and in these a rise in the region of 30 mgm. could not be regarded as abnormal. In these individuals, however, the criterion of normality is the return of the blood-sugar to its fasting level within 2 hours. Any delay in the fall indicates an abnormality in the carbohydrate metabolism of the body.

In this connection case 41 is of interest. At the end of one hour, an increase of 55 mgm. was obtained, but by the end of the following half-hour, the blood-sugar fell to below the fasting-level. This resembles the "lag" type of curve described by McLean with reference to diabetes, and indicates that the storage mechanism was sufficient, but required an abnormally high blood-sugar concentration for the requisite stimulus.

Another type of abnormal curve is that of Case 38 in which, though the rise was only slightly above the maximum normal, there was marked delay in the fall, and by the end of

3 hours had not regained its former level. This curve also illustrates a third type of abnormality, namely, that in which the maximum rise is not reached till after the first hour. In this series of cases, this occurred twice. There are two possible explanations for this phenomenon. Either it is due to delay in absorption from the alimentary tract, supported by the presence of ascites in case 40, or else the storage mechanism could not keep pace with absorption. In spite of the fact that this type of curve was not obtained in other cases of ascites, the former explanation is the more plausible.

The normal curve may therefore be defined as one in which the maximum rise in the blood-sugar level occurs not later than one hour after ingestion of 40 gm. galactose, the increase does not exceed 30 mgm. per 100 c.c. and the fasting level is regained not later than 2 hours after the commencement.

2. Pathological cases: 5 cases of catarrhal jaundice were examined. All of them gave positive results, the smallest rise being obtained in case 11, which had lasted 4 weeks, and was clearing up. In all cases, also there

was delay in the fall to normal.

This supports the view that catarrhal jaundice is essentially a hepatitis, with profound disturbances of function of the hepatic cells. In the early stages, the blood-sugar rise is always considerable, and decreases as the jaundice clears up (121).

In cholecystitis, normal curves were obtained, the increase ranging from 19 to 29 mgm. All the cases were of a chronic nature, with gall-stones. The findings indicated no disturbance of liver function, although some observers hold that the liver is always affected in this condition.

Three cases of jaundice, following the administration of arsenical compounds in the treatment of syphilis, were examined. It was impossible to determine, however, whether the jaundice was due to the arsenic or the infection. In all cases, the jaundice was very slight, and the blood-sugar fell to normal in 2 hours. The smallest rise was observed in the case in which the jaundice had almost cleared up at the time of examination, thus suggesting a quantitative relation between the intensity of the jaundice and the degree of affection of the liver cells.

Another case, No. 21, developed jaundice while undergoing treatment for syphilis, but a few weeks had elapsed between the last injection of neokharsivan and the development of the jaundice. This case showed the largest increase of the series, while in addition there was prolongation of the blood-sugar curve. This case was probably a true syphilitic hepatitis, though the possibility of a co-incident catarrhal jaundice could not be excluded.

Case 22, suffering from syphilitic pachymeningitis, was receiving atophan gr. 7½ nocte, when the deficiency in liver-function was noted. Although the smallness of the dose rendered the atophan improbable as the cause of the liver affection, in view of the well known toxicity of the drug, this led to its discontinuation.

Three cases of carcinoma of the liver are included. In none of them was the carcinoma considered to be primary. In case 37, the woman died, somewhat unexpectedly, the day after the test was performed. At autopsy, a primary focus in the stomach was discovered, with secondary deposits in the omentum and liver, the latter organ being riddled with nodules, so that very little normal tissue was left. In spite

of this, the liver disturbance tested with galactose was only slight, while there was complete absence of any suggestion of jaundice. This is an important point in the differential diagnosis between cirrhosis and malignant disease of the liver, as in cirrhosis, the disturbance in carbohydrate function is generally more marked.

The case of carcinoma of the head of the pancreas with obstructive jaundice gave a normal curve. Mere obstruction of the bile-ducts does not give rise to disturbance of liver-function at first, but prolonged obstruction eventually affects the liver-cells. This would be an important point if operation were considered in a case of obstructive jaundice. The demonstration of marked impairment of liver function would render the operation extremely dangerous.

The three cases of cirrhosis of the liver all gave positive results, and the test probably has a prognostic value, since those cases which were clinically the most serious gave the most marked results. No. 41, in whom the liver was most enlarged, i.e. in whom regeneration was most active at the time of examination, gave the smallest rise. It would thus seem possible to obtain a normal result in a case of cirrhosis

of the liver in whom regeneration was temporarily sufficient to carry on the normal functions of the liver; when this failed, abnormal results would again be obtained.

In unresolved pneumonia, a positive result was obtained, indicating functional hepatic damage probably due to the toxic action of the exudate in the lung.

The same mechanism is responsible for the positive result in the case of bronchiectasis with consolidation, which was considered to be caused by a pneumococcal infection.

Normal results were obtained in pernicious anaemia and postero-lateral sclerosis. It is stated, however, that in the late stages of pernicious anaemia, a positive result is obtained, due to fatty degeneration of the liver. With the advent of the liver treatment of pernicious anaemia, such advanced cases will probably become very rare.

Three cases of nephritis were examined, and two of them gave markedly positive results, Case 33 giving the second highest increase of the series. That the rise was not due to retention is shown by the high excretions of the galactose in both cases. There appears to be a profound disturbance of the carbohydrate metabolism in these cases, but whether the

defect is in pancreas, liver, muscle etc., is difficult to say at present.

In exophthalmic goitre, positive results were obtained. This is not surprising in view of the frequent occurrence of glycosuria in this condition and marked lowering of the carbohydrate tolerance.

The case of tuberculous peritonitis with ascites gave a normal result. This could be an important differential point in the diagnosis of different forms of ascites.

The two cases of enlarged liver due to cardiac failure gave normal results. Neither of the cases was of long-duration, and it is probable that in chronic venous congestion of long standing impairment of liver-function would occur.

In lymphatic leukaemia, a positive result was obtained. This may possibly be due to the fatty degeneration of the liver met with in this condition leading to insufficiency.

Galactosuria:

In no case examined was sugar found to be absent from the urine, but in two cases the amount present was too small to be estimated. Generally speaking, the amount excreted was

roughly proportional to the height of the glycaemia, but there were several exceptions; cases 17, 18, 20, 22, 23, 37 and 38 illustrate the fact that galactosuria cannot be relied upon to give the information supplied by the blood-sugar curve and may, in fact, give quite fallacious results.

Bauer gives the following figures for the excretion of sugar in the urine.

"Ausscheidungen von 0-1 gm. normal.

Ausscheidungen von 1-2 gm. normal bis hochnormal.

Ausscheidungen von 2-3 gm. schwach bis deutlich positiv.

Ausscheidungen von 3-12 gm. deutlich bis stark positiv.

Elmer and Scheps (117) recorded a number of cases in which there was little or no galactosuria, although the blood-sugar rise greatly exceeded 30 mgm. The above figures support their views. Furthermore, the type of curve given by case 38, in which, although the blood-sugar is only slightly elevated, there is marked delay in the fall, would be entirely missed if the urine alone were examined.

Conclusions:

It must be emphasised that besides the liver, the glands of

internal secretion, notably the pancreas, liver and pituitary and the sympathetic nervous system are all concerned in the carbohydrate metabolism of the body. In addition, it is possible that inherited characteristics play a role in determining the nature of the response to ingested carbohydrate.

The blood-sugar curve represents the sum-total of these influences, and in any given case, in order to implicate a single organ, all the others must first be excluded before a definite diagnosis can be given.

In diabetes, the general shape of the curve may resemble that of advanced liver disease, but in the former case, the fasting blood-sugar is invariably greatly elevated, and glycosuria is nearly always present at some time or other. In addition, clinical signs of diabetes are present.

In hepatic diseases, the most marked results are obtained in those conditions which damage the parenchyma of the liver, catarrhal jaundice giving the highest figures, then cirrhosis and syphilitic hepatitis, while in carcinoma of the liver the curve as a rule is not very greatly elevated. Haemolytic jaundice, as in pernicious anaemia, and obstructive jaundice, as in carcinoma of the pancreas give normal results. In

cholelithiasis and cholecystitis also, normal results are obtained.

Positive results are obtained in nephritis and in exophthalmic goitre.

Kahler and Machold (114) say of this test: "Es muss noch festgestellt werden, das weder das Bestehen von pathologischer alimentärer galactosurie, noch der Nachweiss abnorm stärker Galaktosämie imstande ist, jede Leberfunktionsstörung, sondern mit Sicherheit nur eine Störung der Leberfunktion bezüglich des Kohlehydratstoffwechsels anzuzeigen."

The above results show that the galactose tolerance test is a very sensitive indicator of the functional integrity of the hepatic cells. It is of great value in demonstrating whether the liver is affected, but cannot diagnose which particular disease is present. Carcinoma, cirrhosis and hepatitis can all produce similar effects on the blood-sugar curve. A negative result, though suggestive, is of little practical value. In addition, all conditions affecting the carbohydrate metabolism of the body must first be excluded before the liver can be implicated.

From the point of view of prognosis, the test is of

value in cirrhosis and obstructive jaundice, giving a quantitative estimation of the degree of damage to the liver.

THE VALUE OF HEPATIC EFFICIENCY TESTS.

In the investigation of a case with a view to demonstrating the existence of hepatic deficiency, it is advisable to employ several tests, and not to rely on the results of one alone. The reason for this is the existence of functional "asynergy" of the liver (122). When disease attacks this organ, the various functions are not all equally affected; in fact, it is often found that only one function is markedly affected, while in the others dysfunction is slight or absent.

"La synergie fonctionelle est rare en pathologie hépatique humaine. Moins l'atteinte hépatique est marquée, moins la synergie est complète. Dans la majorité des cas, c'est l'asynergie que l'on observe." (122)

There arises therefore the question "Which of the large number of tests described above should be employed in the detection of insufficiency of the liver?" There is hardly a single test which, when first introduced, was not vaunted as the one which would solve the problem of hepatic efficiency.

In my opinion, a great proportion of them will soon be discarded to that limbo where resides Cammidge's reaction in the urine, etc., and will be regarded as mere clinical curiosities.

The investigation of the pigmentary function of the liver, the response of the liver to the dye tests, and the carbohydrate tolerance tests, are the most useful from the point of view of diagnosis.

The estimation of the bilirubin of the blood, either by the Van den Bergh (quantitative) reaction or the determination of the icterus index, should be performed as a routine measure in every hepatic case where the diagnosis is in doubt.

Of the three dye-tests described above, rose bengal seems to be ideal for determining the chromagogue function, though all of them are still in general use, and the results obtained are comparable. Some observers hold that the results of the dye tests merely give an indication of the patency of the bile-passages, and in reality duplicate the results of the bilirubinaemia test. That this is not so, is shown by the presence of an increased bilirubinaemia in some cases of cancer of the liver, with, very often, absence of retention of rose bengal (123). The value of the dye tests is well

illustrated in the case of a large liver, where the diagnosis lies between cirrhosis and cancer. "La présence d'un taux faible due rose apporte la signature du cancer." (124) The precise function which is investigated by means of the dye tests is still a matter of dispute (125).

The investigation of the carbohydrate function of the liver is a very useful adjunct to the foregoing, and of these tests, the galactose tolerance test is certainly most valuable.

The bilirubinaemia test, combined with the rose bengal and carbohydrate tolerance tests, and the routine examination of the urine and faeces, will give all the essential data obtainable by laboratory investigation.

The induced bilirubinaemia test is at present in the experimental stage. It is possible that in the near future it will become established as a fundamental test of hepatic function.

Laboratory data alone, however, can give no diagnosis in hepatic affections. Acute necrosis, cloudy swelling, fatty degeneration and fibrosis all produce similar effects, disturbance of function. It is only when the laboratory findings are combined with the clinical data, that a diagnosis becomes possible.

The ideal hepatic test is one which would express in a single figure the totality of function of the liver. This ideal must remain unattainable, on account of the multiplicity of functions performed by the liver. We must be content at present with testing each function separately. The results obtained stand in the same relation to the hepatic case, as the demonstration of a systolic murmur does to a cardiac case.

Greatest difficulty is encountered in the very early stage of chronic disease, when very often, only slight, if any deviations from the normal are observed. Negative results in such cases are of no value. Only by repeated applications of the tests at varying intervals of time, combined with clinical observation, can diagnosis and prognosis approach accuracy.

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