host rather than to any direct action of the impurities on penicillin itself. This would explain why the enhancement effect cannot be demonstrated *in vitro*, and rules out any direct synergistic effect of the impurities on penicillin. We were not able to confirm that the enhancement effect of the impurities caused the higher penicillin blood levels reported by Welch (1947).

There are as yet no reliable clinical data to demonstrate that less-pure penicillin (from modern deep culture production) has any therapeutic advantages over pure products. Early favourable reports are based on observations with batches of uncertain penicillin content used in the treatment of venereal diseases, mainly gonorrhoea, which are known to respond even to small doses of penicillin. From the pharmacological point of view all the arguments are in favour of using the purest penicillin. It is better tolerated in high doses by sensitive tissues like those of the central nervous system and eye than are cruder products; it has less often been reported to produce allergic sensitization; and it is more stable.

Summary

It has been found that highly purified samples of penicillin G sodium salt were not so effective as less-purified material in protecting mice against infection with *Bact. typhosum*, but that the difference was not demonstrable with streptococcal infections. A suggestion is advanced that the enhancing effect of "crude" penicillin may be partly due to increased leucocytosis in the peritoneal cavity after injection of the impurities present, and it has been shown that certain colloids do produce this leucocytosis and the "enhancement" of purified penicillin.

REFERENCES

- British Medical Journal* (1948). 1, 795.
Dunham, W. B., and Rake, G. (1945). *Amer. J. Syph.*, 29, 214.
Fischbach, H., Eble, T. E., and Levine, J. (1947). *Science*, 106, 373.
Hobby, G. L., Burkhardt, B., and Hyman, B. (1946). *Proc. Soc. exp. Biol., N.Y.*, 63, 296.
— Lenert, T. F., and Hyman, B. (1947). *J. Bact.*, 54, 305.
Miller, C. P. (1933). *Science*, 78, 340.
— Hawk, W. D., and Boor, A. K. (1948). *Ibid.*, 107, 118.
Olitzki, L. (1948). *Bact. Rev.*, 12, 149.
Pratt, R., Dufrenoy, J., and Strait, L. A. (1948). *J. Bact.*, 55, 75.
Ungar, J. (1944). *Nature*, 154, 236.
Welch, H. (1947). *Proc. Amer. Drug Manuf. Ass. Annual Meeting*, p. 137.
— Randall, W. A., and Price, C. W. (1947). *J. Amer. pharm. Ass.*, 36, 339.

NEEDLE BIOPSY OF THE LIVER WITH SPECIAL REFERENCE TO A MODIFIED GILLMAN TECHNIQUE

BY

RICHARD TERRY, M.D., M.R.C.P.

Medical Chief Assistant, St. Bartholomew's Hospital

In 1833, at St. Bartholomew's Hospital, Stanley punctured the liver in a case of suppurative hydatid disease. Ehrlich (Frerichs, 1884) obtained liver tissue by aspiration through a needle in research on diabetes, and Lucatello (1895) used a similar procedure for diagnosis. These early reports were followed by studies employing variations of the aspiration technique, but they were largely unsatisfactory. The present status of liver biopsy dates from the work of Iversen and Roholm (1939), and since then its value has become widely recognized.

Indications and Contraindications

The following circumstances have been judged to indicate the use of liver biopsy:

1. Jaundice.
2. Hepatic enlargement of unknown cause.

3. Malignant disease when hepatic metastases are suspected.
4. Hepatic disease in which precise diagnosis is otherwise difficult: fatty infiltration, acute, subacute, and chronic hepatitis.
5. Obscure disorders possibly due to systemic diseases accompanied by morbid changes in the liver, when other methods of diagnosis have proved inconclusive: sarcoidosis, amyloidosis, haemochromatosis, Gaucher's disease, abdominal reticuloses, aleukaemic lymphatic leukaemia, glandular fever, kala-azar, and schistosomiasis.
6. Portal hypertension—to determine whether the obstruction is intrahepatic and whether lipotropic therapy is required before operation.
7. Constrictive pericarditis—to determine the extent of hepatic damage before operation.
8. The treatment of patients with fatty infiltration, smouldering infective hepatitis, or insulin-resistant diabetes.

The contraindications may be listed as follows:

1. Haemorrhagic states, failing to respond to therapy.
2. Marked anaemia.
3. Absence of superficial liver dullness.
4. Senility.
5. Lack of co-operation of the patient.
6. Refusal of the patient to enter hospital. Watson (1948) reports that some persons are carrying out liver biopsy as an ambulatory procedure.
7. The suspicion of liver abscess, hydatid disease, or active cholangitis.
8. Absence of specific indication.

Mortality

There is a widespread opinion that liver biopsy is too hazardous to be adopted as a standard investigation, but this seems to be based upon mortality figures which include series before the discovery of vitamin K. The following are the death rates recorded in modern series:

	Deaths	Biopsies
Gillman (1948)	1	1,000
Sherlock (McMichael, 1948)	2	400
Roholm, Krarup, and Iversen (1942)	2	297
Volwiler and Jones (1947)	1	278
Beek and Haex (1943)	0	200
Davis, Scott, and Lund (1946)	0	79
Herrera and Pardo (1947)	0	72
Hoffbauer (1945)	0	65
Baron (1939)	1	48
Beierwaltes and Mallery (1946)	0	30
Total	7 (0.28%)	in 2,469

This mortality should be assessed in relation to the many accurate diagnoses obtained, but for a fuller appreciation it is necessary to examine the individual deaths.

Gillman's patient bled from an aberrant superficial artery in a "huge tuberculous liver," and that author considered that the patient could have been saved if surgery had been available. Both Sherlock's patients died from haemorrhage and had such severe liver damage that puncture only precipitated the inevitably fatal issue. One had acute necrosis, and the other subacute necrosis, general paralysis of the insane, and rectal carcinoma (Dible, McMichael, and Sherlock, 1943). The two patients of Roholm *et al.* (1942) died from haemorrhage, and although a haemorrhagic state existed vitamin K was not given. One had obstructive jaundice due to carcinoma of the head of the pancreas, and the other glandular metastases in the portal fissure. Volwiler and Jones's fatality followed puncture of the right main hepatic vein 4.5 cm. from the surface of a friable amyloid liver; intrahepatic bleeding led to gross splitting of the liver. Baron's patient had metastases in the liver and elsewhere and bled from a tear 1 cm. long; the bleeding and clotting times were not estimated.

Thus these seven deaths occurred where the prognosis was already hopeless, and three might have been avoided had the haemorrhagic state been treated.

There have been other deaths, but they have been omitted in arriving at the percentage of 0.28%, either because they occurred prior to knowledge of vitamin K, as the two deaths in 100 biopsies by Bingel (1923) and the two deaths in 160 biopsies by Olivet (1926), or because they have been reported without reference to total biopsies.

Bingel's two deaths were from haemorrhage. One patient had carcinoma of the stomach with hepatic metastases, and the other pernicious anaemia with only 500,000 erythrocytes per c.mm. In neither was the haemorrhagic state investigated. Olivet's two patients died from haemorrhage: one had extensive liver metastases and the other pernicious anaemia with haemoglobin 15%, and again no examination was made of the haemorrhagic state.

Raby (1944) reported one death from puncture of a superficial branch of the portal vein in a woman aged 79 years with jaundice of obscure origin: 1.5 litres of blood were found in the peritoneal cavity; the clotting time was normal. He had a second death three days after biopsy of a man aged 85 years, at whose necropsy no cause of death was found. He felt that the age of these two patients was of some importance. Hoffbauer (1947) quotes an unreported death due to perforation of the colon: the subcostal approach was used although the liver was of normal size.

Thus since 1939 only three deaths have been reported in patients whose prognosis was not already hopeless: of these, two were in patients aged 79 and 85 years, and the third was associated with questionable technique. However, in spite of these reassuring findings, it is necessary to realize that these figures have been obtained by skilled operators constantly using the procedure and that the casual employment of liver biopsy must lead to disasters.

Choice of Method

Iversen and Roholm (1939) used a trocar and cannula 17 cm. long with 2 mm. bore, ending in three sharp points, with a syringe to aspirate the liver tissue. Their method entailed insertion of trocar and cannula into the liver, removal of trocar, further insertion of cannula, attachment of syringe to cannula, withdrawal of syringe plunger, and finally removal of syringe and cannula. These six manipulations required the presence of the cannula within the substance of the liver for 10-15 seconds, during which the patient had to hold his breath. The syringe and cannula occasionally became disconnected at critical moments and the liver tissue, being sucked into the syringe, often broke up. Sherlock (1945) used a similar instrument, but changed to a smaller bore after 126 biopsies with two deaths.

In the U.S.A. the instruments described by Roth and Turkel (1944) and by Silverman (1938) have been preferred. In the Roth-Turkel method a short trocar and cannula are introduced about 1 cm. into the liver, the trocar is removed, and the tissue is punched out by a second longer cannula passed through the first; finally, suction is applied with a syringe. Adapted to liver biopsy by Tripoli and Fader (1941), the Silverman needle differs from the Roth-Turkel in that the longer cannula is split longitudinally and both halves tend to spring apart. Having punched out the tissue with the split cannula passed through the short cannula, the latter is advanced until both tips are level, and then both are withdrawn together, no suction being necessary. These methods have the disadvantages of undue time spent within the liver, frequent failure, and, except for the Silverman method, fragmentation of the specimen.

Watson (quoted by Greene, 1944) failed to obtain tissue for examination in 40%, Iversen and Roholm (1939) in 22.5%, and Hoffbauer (1947) in 23% of his first 65 attempts. The problem of failure has usually been approached by modifying the end of the cannula; the cannula of Franseen (1941) merely had three sharp teeth instead of the three sharp points of the Iversen-Roholm needle. Herrera and Pardo (1947) describe an ingenious arrangement of thread which cuts through the core at its base.

The instrument evolved by Gillman and Gillman (1945) overcame these various defects, having only 5% of failures. The following report is based upon a modification of their instrument and technique: 72 biopsies have been carried out without complication, and failure has been experienced on six occasions.

Description of Modified Gillman Instrument

The instrument is a needle mounted upon a 20-ml. syringe with a stylet fitted to the plunger (Fig. 1). The incorporation of syringe, needle, and stylet in one instru-

FIG. 1.—The liver biopsy instrument

FIG. 2.—Showing details of construction

ment allows all the manipulations of the Iversen-Roholm method to be carried out in a single brief movement. Fig. 2 gives details of construction. A 2-mm.-bore needle is used as a routine, but when there is any likelihood of undue bleeding one of 1.8-mm. bore is substituted. The needle has a bevelled point which produces a linear incision in the liver capsule, whereas blunt-ended cannulae make a round hole. The whole circumference of the end of the needle is internally bored to give a knife edge. An adjustable depth guard is mounted on the needle. The stylet has a flattened side allowing pressures within the syringe to be transmitted to the needle. The spring catch fitted on the syringe cap engages notches on the plunger rod and maintains the plunger in position after suction is applied. These notches are placed at intervals of 1 cm. and permit variation in length of the liver core.

Method

Since the patient's co-operation is essential, a preliminary visit is made to gain his confidence, and the operation is referred to in vague terms. No biopsy is done in the presence of increased prothrombin, bleeding, or clotting times. Vitamin K is given as a routine on the two days preceding biopsy and longer if necessary; with jaundice, menaphthone 20 mg. is given intravenously, and without

jaundice 10 mg. t.d.s. by mouth. Two pints (1.14 litres) of blood are held available. Morphine, $\frac{1}{4}$ gr. (11 mg.) subcutaneously, is given 30 minutes beforehand and, if the patient is unduly nervous, phenobarbitone 1 gr. (65 mg.) an hour previously.

The biopsy is carried out in the ward. The patient lies supine with his right side close to the edge of the bed and his right hand behind his head. The superficial liver dullness is mapped out and a point selected one space below its upper level, usually the seventh intercostal space, in the anterior axillary line. This point is marked by pressure with the fingernail, and the area is swabbed with ether.

A 10-ml. syringe is used to introduce 8–10 ml. of 2% procaine, at first with a No. 20 needle for the skin weal and then Nos. 14 and 1 for the deeper tissues. Care is taken over infiltration of the diaphragm and liver capsule with the breath held in expiration. The field of operation is then screened from the patient's direct view and the skin incised for about $\frac{1}{2}$ in. (0.8 cm.).

The piston is lubricated with sterile liquid paraffin, the instrument assembled, and smooth working confirmed. This interval allows the analgesic to take effect, gives the patient a moment's relaxation during which he can practise holding his breath in expiration, and permits one to see how long the skin incision bleeds.

The right hand grips the syringe barrel and the left steadies the needle. With the bevels of needle and stylet in apposition, the point is introduced through the skin incision and advanced with a rotary movement. A "give" indicates penetration of the intercostal space, and, with the patient holding his breath, the point is advanced until liver resistance is felt; the point is then retracted just clear of the liver. This manoeuvre allows one to judge the thickness of the parietes and to adjust the depth guard, and is especially important with ascites.

After further breaths the patient is told, "Breathe in, breathe out, stop breathing." ("Hold your breath" usually produces a further inspiration.) The biopsy is then carried out. The point is first advanced about 1 cm. into the liver, and then, with the left hand gripping the syringe handle and maintaining its position (and therefore the position of the stylet), the needle is advanced *sharply* into the liver by thrusting the syringe barrel forwards with the right hand. These are precisely opposite movements to those used in aspirating an abscess, when the barrel is held stationary and the plunger withdrawn. The barrel is advanced an average of 3 cm., which determines the depth of penetration into the liver. The left hand moves to the front of the barrel, and the whole syringe is quickly rotated through 360 degrees and immediately withdrawn.

The core is gently extruded into a glass tube 5 cm. long and of 3 mm. bore, and placed in 10% formol-saline. A dry dressing covers the skin incision.

Aftercare.—The patient remains semi-recumbent for six hours and in bed until the next day. Pulse readings are taken every 15 minutes for two hours and hourly for six hours. The general condition is closely watched. A mild analgesic is given one hour afterwards and a hypnotic in the evening.

Comment on Method

The intercostal approach has been adopted because it appears safe and easy provided the superficial liver dullness is carefully recorded. The costo-xiphisternal angle approach of Gillman and Gillman has not been used because most patients dislike punctures near the praecordium, and, while it is clearly a safe method in their hands, the inability to map out the liver in this area lessens one's confidence. Gillman and Gillman have expressed their fear that the intercostal route might lead to haemorrhage from

diaphragmatic vessels, but if the core is punched out beyond the liver capsule this risk must be small. Davis *et al.* (1946) advise the subcostal approach because of the danger of tearing the liver should the patient breathe with a needle fixed in an intercostal space, but the abdominal wall appears to grip the needle enough to tear a soft liver, while livers of normal consistency are unlikely to tear. The subcostal route has led most frequently to perforation of hollow viscera; Baron (1939), using this route, has reported a fatality from a tear of Glisson's capsule. The progress of the needle through the rigid resistant intercostal space is more easily judged and controlled than through the soft resilient abdominal wall. There is no need to restrict biopsy to patients with hepatic enlargement when the intercostal route is used.

The procedure should be painless. With imperfect analgesia, pain radiates to the right shoulder and epigastrium. General anaesthesia was employed only once, and it proved impossible to assess the patient's condition afterwards. The skin incision eases introduction of the cannula, avoids confusing the pathologist with skin fragments, and allows recognition of any bleeding state.

Failure may occur either because the core remains attached at its base or because the needle passes between the liver cells without punching out a core. Experiments on cadavers, passing the needle through the entire thickness of the lower part of the liver to eliminate the former possibility, demonstrated that failure always followed a slow passage of the needle, but when it was introduced rapidly a core was always obtained. Thus success depends upon thrusting the needle into the liver sharply, and it is useless to proceed unless the instrument is working smoothly. The cutting edge of the needle must be kept sharp. Removal of the core from the deeper part of the liver avoids leaving a bleeding gap in the capsule or cutting a hole in the diaphragm, and rules out misleading subcapsular histology.

Gillman and Gillman advise full withdrawal of the plunger after the needle has been advanced. This must stretch and may break the core; it adds a further manipulation and has not been found necessary. Nor has their method been followed of rotating the barrel on the plunger, rather than the whole syringe, through 360 degrees, as the rotation is more quickly achieved with both hands on the barrel. Since the stylet remains within the needle the core is not sucked into the syringe and so escapes damage.

The time spent by the cannula within the liver is seldom mentioned in the literature, but Volwiler and Jones (1947) recommend aiming at less than 10 seconds. Gillman and Gillman give 15 seconds, but this includes penetration of the abdominal wall. Stopwatch timing of the present method has given times of 1.5 to 3.5 seconds within the liver.

Difficulties.—Women are more apprehensive than men, and present extra difficulty owing to their deeper subcutaneous tissue and the frequent need to retract the right breast. Cirrhosis has special problems. A shrunken liver may be difficult to locate. Distortion may swing the gall-bladder into a vulnerable position. Fibrosis is the cause of many failures and ascites is a well-recognized obstacle, but they have not caused failure in the present series. Finally, the histology of the core may be normal in the presence of gross cirrhosis, a limitation to which Himsworth (1947) and Learmonth (1947) have drawn attention. The six failures in the present series were attributed to instrumental problems in four cases and inexperience in two; in all six patients the biopsy was successfully repeated.

Inspection of the Core.—The small glass tube protects the core, keeps it straight, and facilitates naked-eye

examination. In the present series the core from each case of obstructive jaundice has shown easily visible green spots scattered throughout, while in each case of parenchymatous jaundice the core has been uniformly coloured. Fragmentation of the specimen is suggestive of cirrhosis and has been observed where the histological report was normal, gross cirrhosis being found at operation for porto-caval anastomosis. Metastases show as pale homogeneous areas, and are found more often than might be expected; foci of lymphadenoma have the same appearance. The greasy appearance stated to characterize fatty livers has not yet been observed. Apart from these conditions, considerable variation in the appearance of the core is compatible with normal histology.

Sequelae

There is often slight aching in the right hypochondrium, and occasionally pain in the right shoulder. This pain seldom lasts more than 24-36 hours and causes no distress. Of the present 72 biopsies 47 have had this reaction. 21 cases denied any pain. In four there was more severe pain, starting suddenly 1-8 hours after biopsy; but no anxiety was caused, and one patient readily agreed to a further biopsy. No other complications have arisen. Chest radiographs of ten patients have shown neither pleural effusion nor pneumothorax.

Complications reported in the literature are infection, perforation of a hollow viscus, and haemorrhage. Infection has been rare. Perforations of the colon, duodenum, and gall-bladder have been reported, usually without any reaction, though non-fatal biliary peritonitis and one death from colonic puncture are recorded. Haemorrhage has followed a tear of the capsule or of a superficial hepatic vessel. Haemothorax requiring blood transfusion has been twice noted.

Summary

Some indications for liver biopsy are given.

A mortality of 0.28% is presented, occurring in 2,479 biopsies reported since 1939, and the published deaths are examined in detail.

A description is given of a method of liver biopsy which has been employed in 72 biopsies with neither death nor significant complication.

I am indebted to Dr. R. Bodley Scott for his encouragement and ready assistance; to Dr. Geoffrey Bourne and the physicians and surgeons of St. Bartholomew's Hospital who have allowed me to carry out needle biopsy on their cases; to Mr. W. J. Ward, curator to St. Bartholomew's Hospital, and Mr. W. T. Harris, of W. H. Bailey and Sons, for their help in producing the biopsy instrument; to the photographic department of St. Bartholomew's Hospital for the illustration; and to the nursing staff of St. Bartholomew's Hospital for their careful reports.

REFERENCES

- Baron, E. (1939). *Arch. intern. Med.*, **63**, 276.
 Beek, C. van, and Haex, A. J. C. (1943). *Acta med. scand.*, **113**, 125.
 Beierwaltes, W. H., and Mallery, O. T. (1946). *Univ. Hosp. Bull., Ann Arbor*, **12**, 13.
 Bingel, A. (1923). *Ver. dtsch. Ges. inn. Med.*, **35**, 210.
 Davis, W. D., Scott, R. W., and Lund, H. Z. (1946). *Amer. J. med. Sci.*, **212**, 449.
 Dible, J. H., McMichael, J., and Sherlock, S. P. V. (1943). *Lancet*, **2**, 402.
 Franseen, C. C. (1941). *New Engl. J. Med.*, **224**, 1054.
 Frerichs, F. T. (1884). *Ueber den Diabetes*, Berlin, p. 272.
 Gillman, T. (1948). *J. Amer. med. Ass.*, **137**, 243.
 — and Gillman, J. (1945). *S. Afr. J. med. Sci.*, **10**, 53.
 Greene, C. H. (1944). *Arch. intern. Med.*, **73**, 349.
 Herrera, G., and Pardo, V. (1947). *Arch. Path.*, **44**, 393.
 Himsworth, H. P. (1947). *Lectures on the Liver and its Diseases*, Oxford.
 Hoffbauer, F. W. (1945). *Lancet*, **65**, 246.
 — (1947). *J. Amer. med. Ass.*, **134**, 666.
 Iversen, P., and Roholm, K. (1939). *Acta med. scand.*, **102**, 1.
 Learmonth, J. R. (1947). *Ann. roy. Coll. Surg.*, **1**, 299.
 Lucatello, L. (1895). *Lav. Congr. med. interna*, p. 327.
 McMichael, J. (1948). *J. Amer. med. Ass.*, **137**, 234.
 Olivet, J. (1926). *Med. Klinik*, **22**, 1440.
 Raby, K. (1944). *Nord. Med.*, **24**, 2161.
 Roholm, K., Krarup, N., and Iversen, P. (1942). *Ergebn. inn. Med. Kinderheilk.*, **61**, 635.
 Roth, A. A., and Turkel, H. (1944). *J. Urol.*, **51**, 66.
 Sherlock, S. (1945). *Lancet*, **2**, 397.
 Silverman, I. (1938). *Amer. J. Surg.*, **40**, 671.
 Stanley (1833). *Lancet*, **1**, 189.
 Tripoli, C. J., and Fader, D. E. (1941). *Amer. J. clin. Path.*, **11**, 516.
 Volwiler, W., and Jones, C. M. (1947). *New Engl. J. Med.*, **237**, 651.
 Watson, C. J. (1948). *J. Amer. med. Ass.*, **137**, 242.

SEVERE RENAL FAILURE AFTER ADMINISTRATION OF APPARENTLY COMPATIBLE BLOOD

BY

A. C. BUCHAN, F.R.C.S.Ed.

Plastic and Jaw Injury Unit, Bangour Hospital, West Lothian

AND

JOHN WALLACE, M.B., Ch.B., B.Sc.

Glasgow and West of Scotland Blood Transfusion Service

The literature on the Rh blood group is vast, but the recent appeal (*British Medical Journal*, 1948, **2**, 344) to publish observations on this subject has prompted us to record the case given below.

The development of irregular haemagglutinins after repeated blood transfusions is now well recognized. Callender and Paykoç (1946) reported that, out of 15 Rh-negative patients transfused with Rh-positive blood, only one developed rhesus antibodies, and Gunz (1946) observed that out of 10 Rh-negative patients who received multiple transfusions two were found to have developed antibodies. These observers point out, however, that had the tests for blocking antibodies (Race, 1944; Wiener, 1944) been performed the percentage of sensitized patients might have been higher. This suggestion is confirmed by Diamond (1946), who in a series of 2,500 Service men transfused under wartime conditions was able to demonstrate rhesus antibodies in the serum of about 50% of the Rh-negative individuals. Hattersley (1947), in a similar but smaller series, demonstrated rhesus antibodies in 11 (55%) out of 20 Rh-negative cases transfused under Service conditions. In all Hattersley's cases the antibodies were in the "blocking" or "incomplete" form, and direct compatibility tests of the patient's serum against saline-suspended Rh-positive cells would not have shown agglutination. However, in spite of this apparent compatibility the transfusion of Rh-positive blood to such a patient would result in a haemolytic reaction. It is for this reason that the following case is presented.

Case Report

A corporal aged 24 received a severe facio-maxillary injury on Jan 1, 1945. At that time he was given 5 pints (2.84 litres) of Group O blood in the course of 36 hours. His Service records make no mention of any reaction to these transfusions.

On July 24, 1946, a bone-graft operation was performed by Mr. A. B. Wallace. During the operation the patient's condition deteriorated, but a satisfactory rise in blood pressure followed the transfusion of 2 pints (1.14 litres) of Group O blood which appeared compatible on direct matching with the patient's serum.

On the morning of July 25 his condition was poor, his pulse rapid and of small volume, and his blood pressure 88/50. His collapse was attributed at the time to post-operative shock. One pint (570 ml.) of plasma and 1 pint of blood were given, and within four hours his general condition had improved, the blood pressure being 106/68. This latter blood was Group O, and appeared compatible with the patient's serum.

On the 27th he complained of nausea and vomiting, and his urine was blood-red in colour. The microscopical report