ASSOCIATION OF CLINICAL PATHOLOGISTS: 48th GENERAL MEETING

The forty-eighth general meeting of the Association of Clinical Pathologists was held in Portsmouth from April 3 to 5, 1952.

Summaries of the papers read at the scientific sessions follow.

Professor D. S. Russell, M.D., D.Sc., LL.D., F.R.C.P., took the chair at the first session.

Pre-juvenile Amaurotic Idiocy

Dr. H. S. Baar reported on the histopathological findings in the extremely rare pre-juvenile or late infantile form of amaurotic idiocy. The illness started at the age of 4 years and the child died when 10 years old. A brief summary of clinical findings was given. The main post-mortem findings were atrophy of the brain which affected equally cerebrum and cerebellum, patchy chronic leptomeningitis, laminar spongy devastation mainly in layers 3 to 5, disappearance of nerve cells, inflation of remaining nerve cells by a granular material which took most of the lipid stains and was insoluble in alcohol, ether, chloroform, and benzene. The granules stained blackish-brown with Weigert's haematoxylin, but not by the Smith-Dietrich method. With the former stain some nerve cells appeared to be replaced by mulberry-like clumps of granules. The variations in staining properties of different nerve cell types and of compound granule cells and in their behaviour in polarized light were stressed. The nerve cell changes were ubiquitous but mildest in the spinal cord, severest in pyramidal and Purkinje cells. Swelling and abnormal ramification were most marked in apical dendrites of Purkinje cells, where a club-like swelling filled with lipid granules was sometimes larger than the cell-body. Gliosis of grey matter was diffuse, but varied in density. Intracellular neurofibrils were pushed to the periphery or absent. Axons were sometimes swollen but did not contain lipids; their neurofibrils appeared to undergo granular disintegration. Demyelination was severe at the hilum of the dentate nucleus of cerebellum, marked in the cerebral cortex and in the immediately subcortical white matter, mild in deeper layers. Some genetic aspects were discussed.

Circulatory Changes in the Liver in the Newborn

Dr. J. L. Emery said that the left physiological lobe of the liver in the newborn occasionally appeared deep red and slightly shrunken. The left and right lobes of the liver of 100 newborn children were examined histologically. Approximately one in three cases of children dying in the first two days of life, one in six in the first fortnight, and one in 10 in the first two months show a relative shrinking in the cells of the left lobe. This asymmetrical change was not seen in the stillborn children. The incidence of alteration in the left lobe of the liver followed that of patency of the ductus venosus. The lesion may be due to a temporary shunting of the blood from the left portal vein through the ductus venosus.

Diagnostic Value of Dudgeon's Rapid Smear Technique

Dr. Gibson emphasized the importance of speed in transferring the smears to the fixative because drying destroys the nuclear pattern. Lantern slides of photomicrographs of the smears were shown to illustrate the appearances of benign and malignant cells.
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At the first session on April 4 the chairman was Dr. E. M. Darmady.

The Value of the Rib Biopsy in Marrow Disorders

Drs. M. S. R. Hutt, P. Smith, and J. L. Pinniger reported that rib resection had been found to be a valuable method of obtaining bone marrow for histological examination during life.

The procedure had been used in 12 patients and had always yielded excellent marrow sections. The operation was simple and no serious post-operative complications had been encountered.

Further Observations on the Vitamin B₁₂ Concentrations in the Serum and Urine of Patients with Megaloblastic Anaemias

Drs. D. L. Mollin and G. I. M. Ross reported Euglena assays of vitamin B₁₂ in serum of 57 patients with megaloblastic anaemias treated with B₁₂, folic and folic acid. The average duration of raised concentrations after B₁₂ injections appeared proportional to the amount retained. Urinary excretion of B₁₂ by these patients and normal subjects was similar after injections.

Atypical Congenital Haemolytic Anaemia

Drs. J. V. DACIE, P. L. Mollison, J. G. Selwyn, and Nancy Richardson reported that of the surprisingly large number of different types of congenital haemolytic anaemia, the best known were hereditary spherocytosis, Mediterranean anaemia, and sickle cell anaemia. Other types existed and six less well-known types were mentioned.

Twelve families in whom atypical cases had occurred were studied. The disorder was classified as non-spherocytic in type in five families; as a hypochromic type distinct from Mediterranean anaemia in one family; as variants of hereditary spherocytosis in two families; as a macrocytic type with leg ulceration in one case; as variants of familial elliptocytosis in two families; and as a type associated with thrombocytopenia and "triangular" red cells in one case.

The case histories of six patients were referred to and their very varied blood pictures illustrated by photomicrographs. The results of osmotic and mechanical fragility tests and of studies of the rates of autohaemolysis were also given.

The effect of splenectomy was variable; in four patients of the non-spherocytic type the operation was a failure, and this was also true of the patient belonging to the macrocytic type and of the patient with thrombocytopenia and "triangular" red cells. In patients with variants of hereditary spherocytosis and familial elliptocytosis respectively splenectomy was definitely beneficial.

The chairman at the second session on that day was the President, Sir Lionel Whitby, C.V.O., M.C., M.D., F.R.C.P.

Compatibility Tests Preceding Transfusion

Dr. P. L. Mollison said that a compatibility test must ensure, first, that there will be no rapid destruction of transfused red cells by iso-antibodies. This may be described as the immediate risk of incompatibility and, in practice, it lies almost entirely in transfusing blood of the wrong ABO group. However, in a special group of recipients, namely those who have had previous transfusions or women who have had pregnancies, there is a definite risk of trouble from antigens outside the ABO system and, above all, from the Rh antigen (D). Secondly, a compatibility test must ensure that there is no likelihood that the transfusion will sensitize the patient to a blood group antigen so that a subsequent transfusion or pregnancy provokes antibody formation. In practice this risk lies almost entirely in transfusing Rh-positive blood to an Rh-negative subject.

A suitable matching test is a protection only against the immediate risks of transfusion. The following technique is recommended.
A 2% suspension of the donor's red cells in 30% albumin is made, and one small volume added to an equal volume of the recipient's serum in a tube. The mixture is left for a minimum of 10 minutes; the red cells are spun down, then the deposit is examined under the low power of a microscope. This test is better than any slide test, since it is not possible in a slide test to provide optimal conditions for detecting both weak agglutinins and weak incomplete antibodies.

The tube test in albumin should be supplemented by the indirect Coombs test in recipients who may have been previously sensitized by transfusions or pregnancies.

To guard against sensitizing recipients to foreign antigens it is sufficient to ensure that Rh-positive blood is not transfused to Rh-negative subjects. Whenever time permits, the Rh-group as well as the ABO group both of donor and recipient should be determined before transfusion. If no tests can be done all women must be assumed to be Rh negative until proved to the contrary.

Errors in the Cross-matching Test

Dr. F. Stratton, Manchester, spoke on the subject of errors in the cross-matching test. These were divided into two varieties: false positive and false negative. False positive ones consisted of the well-known trio of rouleau formation, cold agglutination, and infection, together with certain clotting difficulties.

A recognition of rouleau depended first upon its microscopical appearance, which in saline agglutination is distinctive and recognizable, and secondly upon the fact that the patient's serum in a dilution of 1:3 loses its rouleau-forming properties. In certain cases, such as multiple myelomatosis, rouleau may be very marked.

Cold agglutination is due to true agglutination present only at room temperature and not at 37°C.

False positive results due to infection present as an agglutination at room temperature but not at 37°C. Here, the purple appearance of the cells and the smell of the blood sample may assist in the diagnosis. It is noted that the control tube containing patient's own cells and patient's own serum is valuable in diagnosing false positive errors and in distinguishing one from another.

False negative results are due to zoning, especially in the albumin test, and to haemolysins in the case of anti-A and anti-B antibodies.

An insensitive technique is a major cause of such error, and the tube technique was recommended as the only one suitable for use in the cross-matching test. The albumin test was considered to be the one that must be used if a single test only could be employed, but the difficulties of the albumin technique were enumerated and it was suggested that, by preference, a saline agglutination test between the donor's cells and the recipient's serum should be carried out at room temperature and 37°C, and a Coombs test at 37°C should also be performed.

Blood Transfusion during 1951 in a Hospital Group of 700 Beds

Dr. G. H. Tovey said that a survey of the use of blood, plasma, and dextran in a hospital group of 700 beds during 1951 showed that 1,039 patients were transfused with a total of 2,463 pints of blood, 110 pints of plasma, and 169 pints of dextran. The ratios of pints of blood transfused to number of beds per main department were: medical, 4.5:1; surgical, 5.8:1; obstetrical, 2.5:1; gynaecological (principally abortions), 7.8:1; paediatric, 1.1:1; general practitioner, 0.9:1. Adverse reactions to the fluids transfused were few, the only adverse reactions to dextran being a rigor in one patient and urticaria in another. Both patients had received blood in addition to dextran. Restoration of blood volume was satisfactorily accomplished by dextran transfusion.

A major problem was the excessive amount of blood requested to be matched and held in the blood bank for patients undergoing operation. On an average 28 pints of blood were matched unnecessarily each week from this practice, and a detailed analysis showed that in a limited number of major surgical procedures only was blood transfused with a frequency greater than 30%. Blood was now matched only for patients undergoing more extensive operations, or if the patient had a history suggesting that iso-immune antibodies might be present in the serum. Otherwise the patient's ABO and D-group were determined preoperatively, but no matched blood was held.
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The afternoon was occupied by demonstrations, and for the third session Dr. R. W. Fairbrother, D.Sc., F.R.C.P., took the chair.

Electrolytes and in vitro Bacteriostatic Activity

Dr. B. W. Lacey said that the effect of replacing sodium chloride by any of 30 salts or salt mixtures on the sensitivity of eight organisms to six bacteriostatic agents had been tested by a filter paper disc method on a medium containing agar, starch, glycerol, alanine, glutamate, cysteine, salts equivalent to 0.7% sodium chloride, and 3% horse blood. The salts were the chlorides, sulphates, acetates, malonates and aconitates of lithium, sodium, magnesium, and ammonium. The organisms were all normal or pathological pharyngeal flora: a diphtheroid, Staph. pyogenes, a penicillin-resistant Staph. pyogenes, Strep. faecalis, a paracolon, H. influenzae, H. parapertussis, and H. pertussis. The bacteriostats were bacitracin, penicillin, aureomycin, streptomycin, chloramphenicol, and 4:4 diamidino-diphenylamine (M and B 938). The susceptibility of bacteria and bacteriostatic agents to changes in the ionic composition of the medium increases in the order of listing above. The effect of a given ion varies both with organism and inhibitor. With chloramphenicol and H. pertussis at least, the effect of a cation may also vary with the anion. At times the effect of a given ion on bacteriostatic activity largely reflects the action of the ion on the growth of the organism. Thus lithium markedly diminishes the growth and increases the sensitivity of Strep. faecalis to all bacteriostatic agents. At others the ionic effect may be independent of any obvious effect on growth. Thus sulphate markedly decreases the sensitivity of most organisms to M and B 938 without any change in colony size. The differential effect of ions on the sensitivity of different organisms had been exploited in the development of a selective medium for H. pertussis. Media with different salt content from those in use at present might be found to give a better in vitro estimate of the therapeutic value of some substances, especially perhaps of chloramphenicol and diamidines.

The Selective Distribution of Certain Antibiotics in Animal Organs

Dr. J. Ungar said that, since Florey et al. (1946) had shown that penicillin could be detected in wound exudates for a considerably longer period than in blood, laboratory evidence had substantiated this finding. The distribution of injected penicillin esters, of streptomycin, and of aureomycin in normal and inflamed tissue, particularly in infected lungs, had also been investigated. It was seen that penicillin salts were retained in any inflamed tissue, whereas penicillin esters were concentrated to a higher degree in inflamed lung tissue only. Streptomycin was also retained in inflamed tissue, and aureomycin, in addition, was concentrated in inflamed lungs and even more so in the normal liver.

Observations on Specific Serological Types of Bact. coli in Children

Dr. G. T. Stewart reported that between November, 1951, and February, 1952, 250 cases of non-specific enteritis in young children were investigated. Specific Bact. coli was isolated from only 17 (6.8%), a figure which did not differ significantly from the figures for strains of Proteus and paracolon bacilli in the same group. The numbers of isolations for specific Bact. coli, as for Proteus and the paracolon bacilli, were significantly higher in children under 2 years of age than in older children. A significantly higher proportion (30%) of isolations of specific Bact. coli was made from a group of 40 symptomless children, of comparable age groups,
who were contacts of some of the cases of enteritis from whom the specific strains had been recovered.

Analysis of the strains recently isolated showed that type 055 (Aberdeen β) was most common. In 1950 and the earlier part of 1951 this strain was uncommon in the Paddington-Marleybone area.

Two of the most severe cases observed in the series, both fatal, were described. One patient died with haemorrhagic pneumonic lesions from which Bact. coli type 026 was isolated; the other died with signs of septicaemia, Bact. coli type 055 being isolated from the blood.

It was concluded that specific strains of Bact. coli were not at present commonly associated with enteritis in London children. Older children, and contacts of cases, might harbour large numbers of the organisms without disease, but the potential pathogenicity of some strains, in children under 2 years of age, was very high, and not necessarily limited to the gastro-intestinal tract.

The Incidence and Demonstration of Biological Reactions in Routine Wassermann Reactions

Dr. H. M. Rice said that many non-syphilitic, positive Wassermann reactions could be distinguished by the Harrison-Wyler-Lacey method (dilute-antigen row added to the standard test). Sensitivity and specificity were both increased. With the dilute antigen, weak sera showed less, and biological sera more, haemolysis than with the standard suspension. The results of 15,870 consecutive routine Wassermann reactions, including minimal reactions normally disregarded, showed 263 frank biological reactions (1.66%), 69 positives (0.43%) and 194 doubtfuls (1.22%). Females showed 43 positives (0.57%) and 119 doubtfuls (1.50%) in 7,479 tests (total 162 = 2.16%); males showed 26 positives (0.31%) and 75 doubtfuls (0.89%) in 8,391 (101 = 1.20%). Hospital cases gave: female, total 71/5,228 (1.36%); male, total 42/4,401 (0.95%); V.D. clinics 91/2,251 female (4.04%); and 59/3,990 male (1.43%).

Some biological reactions were masked, i.e. of syphilitic reaction type. To obtain comparative figures all positive and doubtful reactions were analysed, and final diagnoses determined. Results of syphilis cases were excluded, though sero-positive cases may sometimes show biological reactions. Total biological reactions, including repeat tests, were 325/15,870 (2.05%); positive 93 (0.59%), doubtful 232 (1.46%).

Some doubtfuls were only detectable by the dilute antigen test, being negative to the standard tests. Detailed analyses were shown. Frank biologicals comprised the following percentages of all biological reactions: female, V.D., positive 100, doubtful 95; hospital, positive 56, doubtful 82; male, V.D., positive 59, doubtful 79; hospital, positive 69, doubtful 75; overall total for series 81. Simple dilute antigen techniques were, therefore, most useful for distinguishing biological from syphilitic reactions, in addition to increasing both sensitivity and specificity.

On April 5 the chairman was Dr. E. N. Allott, F.R.C.P.

The Level of Plasma Amino-acids and Phenols in Liver and Renal Disease

Drs. E. M. Darmady, J. A. Durant, J. Harkness, and Patricia A. Loud reported that in studying acute renal failure with uraemia it soon became clear that the blood urea did not accurately indicate the degree of toxicity, and in view of the success of the high calorie diet which was stated to prevent the endogenous breakdown of protein and the overproduction of amino-acids, it was surprising to find that a number of cases showed no increase in amino-acids either in total amount or in abnormal distribution.

An investigation was therefore made into 78 cases, 36 of which showed kidney failure and/or uraemia. As a result it was found that although the blood urea was raised, the level of phenols was more in keeping with the symptoms of uraemia. On the other hand, in studying the toxicity of liver failure the phenol levels were also frequently found to be normal but the total of amino-acids was raised. Before undertaking this work the normal levels of both amino-acids and phenols were investigated in 30 normal cases. These ranged from 0.9 to 2.5 phenols and 4.2 to 7.2 amino-acids.
Single Injection Methods for Renal Clearance Tests

Dr. C. P. Stewart described formulae developed in his laboratory (Robson, Ferguson, Olbrich, and Stewart, 1950) which permitted the measurement of inulin, diodone, or p-amino-hippuric acid clearances after a single intravenous injection of a carefully measured amount of the test substance and without catheter collection of urine. After the injection two samples of blood are required and, at the same (widely spaced) times, two samples of urine with complete emptying of the bladder. The urine samples are required to measure the amount of test substance excreted up to the time of their collection; the second one also gives the volume of urine excreted during the test period (Olbrich, Ferguson, Robson, and Stewart, 1950).

The formulae avoid many of the fallacies of previous single injection methods, since they do not assume equilibration between plasma and extravascular fluids. Rapid and violent fluctuations of water distribution might interfere, since the formulae give "average clearance" over 60 to 100 minutes, but in practice this is not a serious objection. Nor, because of the long test period and the slow change in plasma concentration, is any serious error introduced because of slow mixing in the collecting tubules. The use of venous blood for the analyses, for substances which, like diodone, show a considerable arteriovenous difference in concentration, involves the development of a new normal range. For inulin clearance the new normal is the same as for the infusion method, for diodone clearance it is 20% lower, and for TmD 8% lower. With this correction the formulae provide clearance figures with as great accuracy as is given by clearance methods, and with a very considerable simplification of technique as well as a reduction in the analytical work.

REFERENCES


The Comparison of Radioactive and Metabolic Methods of Thyroid Investigation

Drs. J. B. Foote, D. H. Mackenzie, and N. F. Maclagan reported that the degree of correlation between the basal metabolism and the thigh-neck clearance (Foote and Maclagan, 1951) had been examined in 162 cases mostly suffering from various disorders of the thyroid gland. One hundred and forty of these were untreated and 22 had received treatment with various drugs. There was a fairly close correlation (correlation coefficient = 0.76±0.035) in the 140 untreated cases, but those treated with iodine, thiouracil, or thyroid extract showed a gross discrepancy between the two tests and fell well outside the main correlation area. These deviations are summarized in the following table. The high degree of correlation between the two tests in untreated cases may be taken as confirmation of the value of the thigh-neck clearance in the assessment of thyroid activity. In treated cases the radioactive test is of limited value.

REFERENCE


The Nature of Complement

Professor J. R. Marrack said that Heidelberger (1941) showed that antigen-antibody precipitates formed in the presence of fresh guinea-pig serum contained more N than precipitates formed in presence of saline or of heat inactivated serum. He inferred that this extra N was the N of complement. Similar amounts of N are added to antigen-antibody precipitates from fresh human, ox, and pig serum. These sera contain widely different amounts of complement and of components of complement, measured by ability to promote lysis of sheep red cells sensitized by horse or rabbit antiserum.
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Recently Johns measured the N in precipitates formed by constant antibody with varying amounts of antigen in presence of active and heated human sera. The N added (1) is very small with fresh human sera; (2) is about the same per ml. with fresh human and horse sera as had been found with guinea-pig and pig sera; (3) with heated human sera, varies with the amount added and the amount of antigen and may increase or decrease the precipitate N.

Conglutination is contained in serum of oxen and other ruminants; it is heat stable. It promotes agglutination of particulate antigens treated with antibody and with fresh serum of horses and some other species, supplying conglutinating complement. Conglutinating and haemolytic complements seem to differ in their fourth components. Johns finds that ox serum adds further N to precipitates formed in the presence of both fresh and heated human serum, but not in the presence of horse serum.

Fresh serum appears to contain some protein, the amount of which varies little among the species studied but varies much in ability to promote haemolysis or conglutination in the presence of ox serum.

The final session was presided over by Dr. L. R. Jones.

The Effect of Cortisone Therapy on the Histology of Rheumatoid Arthritic Joints

Drs. D. King and D. Wilson said that five patients suffering from rheumatoid arthritis were selected for this investigation. Three patients were treated with daily intramuscular cortisone, and biopsies of affected knee-joints were performed before the start of treatment, at eight weeks, and at 15 weeks. The remaining two patients served as controls. Dr. Wilson described the clinical features and briefly detailed the clinico-pathological investigations and reactions. The increasing delay in healing of the successive biopsy wounds during treatment was a striking feature. Dr. King drew attention to the inherent difficulties of obtaining comparable biopsies, and demonstrated the variable inflammatory reaction in juxtaposed synovial membrane. He was able to show that after more than three months of treatment the inflammatory reaction was still active and did not reflect the marked clinical improvement.

Barbiturate Levels in Cases of Intoxication

Dr. L. C. Nickolls said that the clinical pathologist is often faced with cases of coma in patients received in hospital, which may be due to barbiturates. Rapid diagnosis is essential.

Barbiturates may be divided roughly into three categories on the basis of speed of action and behaviour in the body as follows:

<table>
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<tr>
<th>Classification of Barbiturates</th>
<th>Speed of Action of Fatal Dose</th>
<th>Behaviour in Body</th>
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<tr>
<td>(2) Medium: phenobarbitone and medinal</td>
<td>Few hours to three days</td>
<td></td>
</tr>
<tr>
<td>(3) Slow: rutonal, barbitone</td>
<td>One to 6 days</td>
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Cases of the first type do not often reach the hospital, since death supervenes so rapidly. The barbiturate level does not exceed 5 mg. per 100 ml. of blood or C.S.F. however great the amount taken, since death occurs as soon as this level is reached or exceeded. Moreover, if the amount taken was a minimum lethal dose so that death is unduly delayed, the body may contain no residual barbiturates at all.

Cases of the second type are commonly received while still comatose and with a good hope of recovery. The barbiturate level is a minimum of 5 mg. per 100 ml., and may rise to double this figure.

Cases of the third type are comparatively rare nowadays, but when they do occur it will be found that the blood barbiturate level is often very high. He had found as much as 25 mg. of barbitone per 100 ml. of blood still circulating several days after ingestion.

The levels given above indicate that with types 1 and 2 a sensitive method of identification is essential. The better modifications of the Cobalt test are sensitive to about 0.1-0.2 mg.

Reference

This means that a minimum of 5 ml. of blood are needed for a satisfactory test. In these circumstances it is obvious that a stomach washout is a more satisfactory sample, since even after some days there is still a minimum of several milligrammes of barbiturate present.

**Necropsy Findings in Three Members of a Family**

Dr. D. E. Price said that in 1951 the deaths of three young children, whose mother was a nurse, were investigated. The deaths occurred over a period of 22 months, and each was inquired into by H.M. coroner before they were considered together.

The first to die was the second child at the age of 4 months. A "general practitioner" finding at necropsy was "bronchopneumonia." The third child died 18 months later when it was 6 months old, a few hours after an operation, as an out-patient, for removal of a naevus from the vulva. A pathologist diagnosed at necropsy "acute heart failure following operation for excision of a naevus with status thymolymphaticus contributing." The cause of death in the case of the eldest child, the last to die, was "barbitone poisoning," and the circumstances suggested that the child had either consumed the tablets herself in mistake for sweets or had been fed with the tablets by another person.

The records showed that barbitone poisoning could not be excluded in the two earlier cases and that on each occasion the only person who had access to the child was the mother.

Exhumation was carried out. The findings were described. There was no evidence of poisoning by barbitone drugs. The findings of the previous inquiries were allowed to stand.

The attention of the meeting was drawn to the following points: (1) the negative character of the findings at post-mortem examinations in cases of barbitone poisoning; (2) the vast use of the barbitone group of drugs and the careless attitude adopted towards them; (3) the danger of post-mortem examinations being conducted by doctors with no forensic pathology included in their training.

**Disposal of the Body by Burying**

Dr. H. S. Holden dealt with two cases in which considerable ingenuity was shown in the disposal of the body of the victim following murder.

In the first of these the body was interred under the brick floor of the coal cellar. To avert suspicion the murderer advertised the victim as missing in both the local press and a widely circulated Sunday paper. He also secured a B.B.C. announcement asking for her return. Intensive police investigation following a study of the "missing persons" file resulted in the discovery of the body 12 months later.

In the second case the murderer confessed the crime committed nine years previously. He had put the body in a sack and buried it in the earth below a large heap of farmyard manure in a field adjacent to a cow byre. Although the site of the dung heap had been cleared long before, he indicated to the investigating officers the actual place of burial and the remains were disinterred and examined in detail.

In both cases the description of the persistent fragments of clothing and the study of the dentures which were recovered proved of material help in identification.