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JOURNAL OF THE NEW ZEALAND ASSOCIATION OF BACTERIOLOGISTS

CONTENTS:

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THE ROUTINE IDENTIFICATION OF CANDIDA (MONILIA) ALBICANS

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Candida albicans is by far the most common yeast to be encountered in medical bacteriology, but in direct smears and upon the usual media used for primary isolations it is not to be distinguished from other, non-pathogenic, Candida. Week-old cultures upon Sabouraud agar have a typical appearance, showing abundant pasty cream growth, sometimes with a greenish cast, and having a strong yeasty smell, but though this is indicative of the nature of the yeast it is not sufficiently exact.

The criteria used for identification are:—

(1) The production of mycelium and chlamydospores (almost always terminal) when the yeast is grown anaerobically upon a starvation medium such as corn-meal agar.

(2) The production of acid and gas in glucose and maltose, the production of a slight amount of acid in sucrose, and no change in lactose.

(3) Pathogenicity for rabbits. This is not a routine procedure but is useful in dubious cases.

Chlamydospore production: The original formula for corn-meal agar used by Benham (1931) is given below, and for a successful outcome it is essential that it be reproduced exactly. Later authorities, reproducing the formula, have sometimes given a single autoclaving for the final sterilisation of the medium, but this has so adverse an effect upon its qualities as to make it almost useless. Practically every worker has his own method of culturing upon this agar, but in this laboratory the simplest and most satisfactory method has been to pour it in Petri dishes, spread the surface of the plate with the yeast under investigation, and incubate it at 27°C in 10% CO_2. After a period of from one to three days, examination of the plate with the low-power objective of the microscope will usually show mycelium and chlamydospores. Sometimes there may be a delay of a week or ten days before chlamydospores appear, but in these cases we find it preferable to rely upon fulfil-

ment of other criteria rather than to delay the report excessively. An incubation temperature of 27°C is ideal, but room temperature or 20°C incubation may be used; the plates should never be kept at 37°C. A McIntosh-Fildes jar may be used for anaerobiosis, but a candle-jar is quite satisfactory.

Various authors have attempted to make generalisations about and draw conclusions from the arrangements of blastospores, mycelium and chlamydospores upon starvation media. What appears to be regarded as the classical picture of Candida albicans under these conditions is tree-like mycelium with numerous terminal chlamydospores arising from colonies of blastospores. In our experience, however, the only typical feature of *C. albicans* upon corn-meal agar, differentiating it from all other species of the genus, is the chlamydospore. The numbers in which this appears varies greatly from strain to strain, and on one or two occasions we have isolated strains which, in spite of all efforts, produced no chlamydospores, although the yeasts in question, by sugar reactions and pathogenicity to rabbits, were undoubtedly *C. albicans*.

Sugar reactions:

Gas appears in the appropriate tubes in two days if they are held at 37°C, but acid production in the sucrose may not be apparent for three or four days. Ordinary bacteriological sugars may be used, but stronger sugar solutions of 2-3%, put up in $\frac{3}{4}$in. tubes, are better. It is important that the final pH of the medium be not greater than 7.2, as a more alkaline reaction masks acid production in sucrose. In the absence of late appearance of chlamydospores, the correct reactions in sugars and the production of mycelium are sufficient evidence that the yeast is *Candida albicans*.

Animal inoculation:

Pathogenicity of the strains of *C. albicans* varies, but as a rule a dose of forty million cells of a one- or two-day culture, given intravenously in normal saline, will kill a rabbit in three to ten days. On autopsy white, pinhead abscesses containing yeasts and mycelium will be found scattered through all parts of the body, but particularly in the kidneys, and to a less extent upon the heart.

Variants of Candida albicans

The yeasts, like other fungi, are "a mutable and treacherous race," and it is to this mutability that some of the appalling confusion in nomenclature is due.

C. albicans var. stellatoidea:

Jones and Martin (1938) reported from America that in cultures from normal vaginas they found a non-pathogenic variant of *C. albicans* characterised by star-shaped colonies on blood agar, mycelium with large, ball-shaped clusters of blastospores and few or no chlamydospores on corn-meal agar, inability to produce acid

in sucrose, and lack of pathogenicity for rabbits. American workers substantiate this finding, but there appears to have been a failure to detect the variant in Great Britain, and in this laboratory no strain has been isolated which agrees with this description. In many instances colonies of *C. albicans* on blood agar have had a star-shaped appearance due to the production of mycelium with balls of blastospores, and mycelium with balls of blastospores, with or without chlamydospores as well, is often seen upon corn-meal agar. Failure to produce acid in sucrose has only been met with in one or two of many hundreds of strains studied, and where this has occurred the other characteristics of the variant have not been present. It therefore appears as if this variant is, if not absent, at least uncommon in this country.

R. variants:

Upon prolonged culture on artificial media usually, but sometimes in primary cultures, roughened colonies, crateriform or folded, with or without a mycelial halo, may appear. Growth is characterised by an unusually large proportion of mycelial elements, in extreme cases to the almost total exclusion of blastospores. The variation is continuous, from strains where the change from the normal smooth colony is so slight as to be almost unnoticeable, to instances where the microscopic appearance is such that it appears as if a wholly different species is being dealt with. With the change to the R form there is a progressive lessening in pathogenicity to rabbits, but the biochemical characters remain unaltered, and the microscopic elements are usually unchanged in quality, but not in their numerical proportions to one another. We have found as a rule that, where the R forms appear in primary cultures, it is only as a small percentage of the total numbers of R and S colonies.

Summary:

Candida albicans may be distinguished from other yeasts by its ability to produce chlamydospores and mycelium under appropriate conditions, and by its ability to produce acid and gas in glucose and maltose and some acid in sucrose. Where chlamydospore production is slow, the presence of mycelium and appropriate sugar reactions may be used as the basis for identification. Pathogenicity of the yeast for rabbits may be used as a further check, but is unnecessary in routine work.

Media

Corn-meal Agar:

Heat at 60°F for one hour 62.5 gm. corn-meal (obtainable in New Zealand from dealers in fowl feed) in 1,500 cc. water. Filter through paper. Make up the volume to 1,500 cc. Add 45 gm. agar. Heat in steamer for 1¼ hours. Tube, or put up in flasks, and sterilise by steaming for half an hour on three successive days.

Dextrose Yeast Extract Broth (Kurung 1942):

Dextrose 8 gms. Sodium chloride 1.7 gms.
Yeast Extract (Difco) 0.5 gms. Distilled water 200 cc.

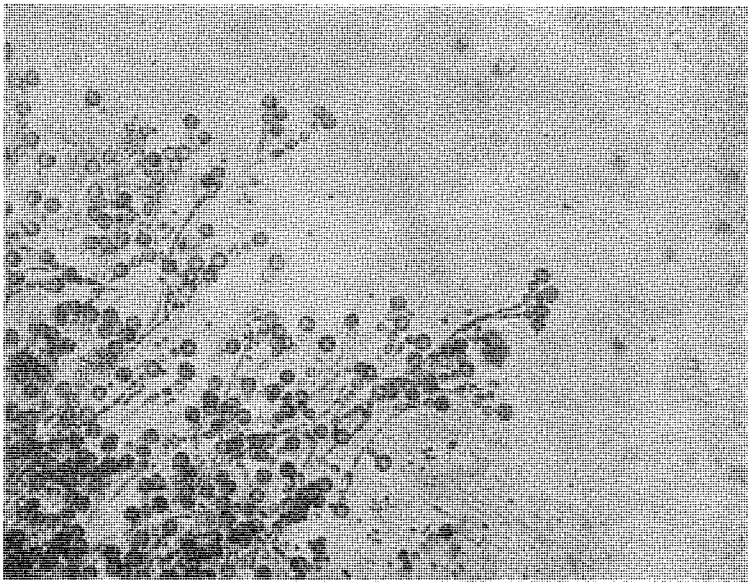
Adjust medium to pH 4 with 1N HCl. Tube, and autoclave at 15lb. for 15 minutes. This acid broth inhibits bacterial growth; subcultures in it of yeasts from contaminated primary cultures are usually pure.

Sabouraud Agar pH 4

Flasks of ordinary glucose or maltose Sabouraud agar are melted and brought to pH 4 with 1N HCl. Plates should then be poured with it immediately. The acid need not be sterilised, but the medium once acidified should not be subjected to further heating. This medium is excellent for the isolation of yeast from heavily contaminated material such as faeces.

References:

Benham, R. W., 1931, J. Inf. Dis., 491, 183.
Jones, C. P., and Martin, D. S., 1938, Am. J. Obstet. and Gynaecol., 35, 98.
Kurung, J. M., 1942, Am. Rev. Tub., 46, 367.



Edge of a colony of *C. albicans* grown upon corn meal agar, showing mycelium and chlamydospores.

High power.

NON-PATHOGENIC ACID FAST BACILLI

By I. W. Saunders

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Our acquaintance with these bacteria, even over many years of experience, tends to be rather restricted on account of the small amount of material available in routine work and partly owing to shortage of literature on the subject.

We meet early with *Myco. smegmatis* in examination of urine for *Myco. tuberculosis* found in the region of the external genitals of both sexes. Although often microscopically indistinguishable from the tubercle bacillus, like many others of the non-pathogenic types, it tends to be shorter and thicker and is generally said to be acid but not alcohol fast. Experience soon teaches us that this is not invariably the case. Usually we can see minor points of difference from tubercle bacilli, which make us suspicious. In catheter specimens of urine we are not likely to encounter smegma bacilli. Difficulty arises when we find them encased in hyaline material or as scattered bacilli resisting decolorisation. Inoculation of the material into a guinea pig will show no lesion after the usual six to eight weeks if we are dealing with smegma bacilli. Further, if we find no growth on ordinary media within forty-eight hours, we are likely to be dealing with tubercle bacilli. Czaplewski was first to cultivate smegma bacilli and obtained growth on a serum medium as yellowish grey colonies, irregularly rounded, sometimes becoming confluent to form a comparatively thick layer. He obtained growth on glycerol agar and in broth. Its growth characters in general resembled those of other members of the group. Only when very large doses are given are smegma bacilli pathogenic to laboratory animals.

Acid fast bacilli are found in secretions of the external genitals and mammae of certain of the lower animals, but vary in character. The term "smegma bacillus" appears to cover a number of related species.

Acid fast bacilli are sometimes found in faeces from healthy individuals, so that it is not safe to report tubercle bacilli present on microscopic examination.

From the above considerations and other matters to be mentioned later, it is obviously wrong to report on microscopic examination alone, tubercle bacilli to be present in any material other than that coming from the interior of the body, such as sputum, serous fluids and pus from newly-opened lesions. That non-pathogenic types are found in these materials very occasionally, need not alter the validity of this statement. Even in the case of gastric lavage it would seem safer to report that "acid fast bacilli" (not tubercle bacilli) were found on microscopic examination. More will be said of this later.

Acid fast bacilli present around the mouths of old water-taps and in distilled water could be a source of trouble in slides stained by the Ziehl-Neelsen method. Similar bacteria are found in the bottoms of old and dirty water-traps used in filtration by suction, and bark corks should not be used in containers for sputum, as they may harbour acid fast bacilli. The older taps in our laboratory appear to contain acid fast bacilli at all times.

That acid fast bacilli are found in grass, butter, dung, water and soil is well known and it is obvious that some of these can be present as contaminants in specimens. Various authors have written of these bacteria, but the literature is not all easily obtained.

Herta Schwabacher (1933) isolated colonies of saprophytic acid fast bacilli from blood cultures from four patients with pulmonary tuberculosis, two with non-pulmonary tuberculosis, and one with schizophrenia. All colonies except in one culture were yellow or orange; the other had whitish colonies which on subculture gave a typically pigmented growth. Time of development of colonies varied, with the shortest nine days. All grew readily at 22°C and all were completely avirulent to guinea pigs, rabbits and mice.

Six similar strains were isolated from 173 bloods from rabbits experimentally infected with tuberculosis. Three similar cultures were obtained from blood of fifty normal rabbits. The conclusion was temporarily drawn that these organisms gained access during manipulation of the blood.

In 81 scrapings from human skin and 56 swabs of sebaceous matter no growth of saprophytic acid fast bacilli was obtained.

In comedones from 19 cases of acne, acid fast bacilli were seen microscopically in two cases, but in cultures no growth of these bacilli was obtained, but in one case using ligroin with a suspension, in broth, a growth was obtained.

Ramel found acid fast bacilli in pus of 9 out of 17 cases of acne.

No acid fast bacilli were isolated from 10 cough plates (Löwenstein's medium) or 225 nasopharyngeal washings on glycerine agar, or from air washed with distilled water planted on Löwenstein's medium or leaving the latter exposed to air.

From 3 samples of street dust, 5 from an animal house and 3 of hay dust, one culture of acid fast bacilli (from animal house dust) and one (from laboratory dust) were obtained.

Films of slime from 56 cold-water taps in constant use showed acid fast bacilli in every instance, some morphologically indistinguishable from tubercle bacilli, others rather stouter. Three hot-water taps seldom used also showed acid fast bacilli. Nine cold-water taps not in constant use, 25 hot-water taps and 25 gas taps were negative.

From 6 cold-water taps, water planted directly on to Löwenstein's medium showed one pigmented colony. Use of a brass screw in tap water with a lump of chalk gave some positive results when incubated at 37°C. for 3 weeks.

Stagnant water, e.g., at the bottom of pressure bottles containing tap-water, old samples of distilled water, and slime from waste overflow pipes of sinks give microscopically positive results, and some smooth pigmented colonies were obtained on culture.

Cultural appearances were much as previously described in earlier work with rough and smooth forms, the bacilli in the former microscopically showing much pleomorphism and in the latter lying more or less parallel to one another and in bundles fairly long, curved, slender, sometimes beaded bacilli, or less often occurring as rather short, stout, sometimes ovoid bacilli staining evenly and arranged singly, in groups or sometimes short bundles.

All strains were strongly acid and alcohol fast. Schwabacher concluded that classification was not possible unless a large number of strains were examined, with particular attention paid to biochemical reactions and serological study.

Gordon (1937) reported an attempt to find standards for classification of rapidly-growing non-pathogenic mycobacteria and presented results of a culture study of 252 strains from soil, plants, human and animal tissues and secretions. This paper could not be obtained for reference. Eighty per cent. were readily separated into three groups of closely-related cultures. The remaining twenty per cent., which differed from the above and from each other, were arbitrarily distributed among the three major groups as subgroups. Since 84% of the cultures had been isolated from soil by means of Söhngren's technique, the question was whether cultures from sources other than soil would fall into the same groups and whether the previous-set standards would be adequate. It was thought that some of the unrelated single strains from the soil collection might be represented in large numbers in cultures from other sources.

Gordon & Hagan (1939) studied 79 additional strains by the same methods as in the previous report. Numbers and sources are given. The fish strains were isolated by Söhngren's technique (1913). Details of this could not be obtained.

Number.	Culture.
11	Human and rat leprosy.
1	Bovine tissue.
1	Sputum.
1	*Saprophytic strain.
10	Spontaneous tuberculosis of killifish (Platypoecilus maculatus).
39	Water in killifish aquaria.
16	Local soil.

* Culture labelled "The original Koch strain of Human Tuber-

culosis." The grouping of these differed slightly from the author's first arrangement.

GROUP I:

Fails to survive at 60°C. for an hour.

Grows at 47°C.

(a) Utilises arabinose, sorbitol, galactose, trehalose, mannitol and frustose (in Merrill's medium). Fifteen strains.

(b) Unable to utilise all the above carbohydrates. Two strains differing in appearance and cultural reactions and from each other.

GROUP II:

Fails to survive at 60°C. for 1 hour.

Does not grow at 47°C.

(a) Unable to utilise sorbitol.

(1) Unable to utilise arabinose. Forty strains similar in appearance and cultural reactions. Two dissimilar strains.

(2) Utilises arabinose. Two similar strains.

(b) Utilises sorbitol. Eight dissimilar strains.

(c) Unable to utilise any of the carbohydrates tested. Ten similar strains.

GROUP Ia: Strains produced a rapid-spreading and wrinkled growth. Growth at first dull, creamy white, deep yellow or orange. All the test carbohydrates except sucrose were utilised. Of the fifteen cultures placed in this group, 10 were soil isolations; one was from sputum of a tuberculous patient, another was the so-called saprophytic tubercle culture; the remaining three strains bore the name *"Mycobacterium leprae."* Two of these were from leprous rats: the third from a culture of Levy-Kedrowski.

GROUP 1b: The group was created for cultures which grew at 47°C. but were not of the myco-smegmatic type. There were four dissimilar cultures, two leprae strains and two soil isolations which had been placed in the group in the previous study. Two more cultures, the leprae strain of Lombardo-Pellagrine and the isolation from bovine tissue were added. The resulting 6 cultures were dissimilar in appearance and biochemical reactions.

GROUP IIa: The predominating culture type of this group produced creamy white growth which did not change colour with ageing. It was either rough or smooth in type; it utilised fructose and in most cases trehalose and mannitol. Forty cultures, one a soil isolation and thirty-nine water strains, were assigned to this group. Another type placed in the group showed the same biochemical reactions as the predominant type, but produced yellow pigment. The remaining dissimilar strain, Myco-leprae of Brinkhoff, is a smooth pale yellow culture which utilised mannitol, trehalose, fructose and sucrose. It was similar to the Duval leprae strain previously included.

GROUP IIa 2: The two smooth salmon pink soil cultures placed in this group utilised arabinose, galactose, mannitol, trehalose and fructose. These cultures resembled none of the seven dissimilar cultures previously assigned to this group.

GROUP IIb: The eight cultures assembled in this group represented six different types. A smooth, yellow soil isolation and the leprae strain of Needham appeared identical in appearance and cultural reactions. They utilised all carbohydrates tested and resembled two soil isolations previously described.

A rough creamy white soil isolation utilised sorbitol, mannitol, trehalose and fructose and was similar to three soil isolations previously included in the group.

The remaining five leprae strains were very nearly identical in biochemical reactions but varied in appearance. The smooth salmon pink leprae culture of Levy-Chione was apparently identical with the leprae culture of Binkerhoff and Clegg previously studied. The red pigmented culture of Rost-Williams resembled a soil isolation in the former collection. The rat leprosy strain of McCoy resembled the leprae strain of Elly previously included in this group. The two pale yellow pigmented cultures of Currie and Barry seemed closely related if not identical with each other.

GROUP IIc: The ten yellow pigmented cultures isolated from spontaneous tuberculosis of killifish, utilised none of the carbohydrates employed. They would not grow at 37°C. nor on Söhngren's medium. They were not identical with any culture in the former collection. Seven of the first cultures, including Mycocheloni and Myco-schlangen, did not utilise any of the seven carbohydrates. These seven cultures were previously placed in Group IIa 1. It seemed advisable to create a new subdivision of Group II and to assign it to these comparatively inert cultures.

The authors gave tables showing data of the cultures. The new plan affected the arrangement of the cultures first studied only slightly.

The collections contained 19 strains labelled "*Mycobacterium leprae.*" These showed considerable variation in appearance and cultural characteristics. Some of them (5) could not be distinguished from certain soil isolations. Ten strains (two pale yellow, four deeply yellow, four salmon pink) utilised sorbitol, but not arabinose or galactose, and did not grow at 47°C. Only 7 of the soil isolations showed this combination, but they did not resemble any of the leprae strains in appearance. Attempts to separate the leprae strains more definitely from the soil cultures were unsuccessful.

The non-pathogenic acid-fast bacilli apparently are antigenically distinct from the tubercle bacillia and are probably antigenically heterogenous among themselves.

A comparison of the chemical composition of human tubercle bacilli, avian tubercle bacilli, bovine tubercle bacilli and timothy grass bacilli shows a tendency to gradation through the group, e.g., per cent

of dried residue is	75.01	83.71	85.50	87.70
Total lipins	23.78	15.26	13.40	8.37
Polysaccharide	0.87	1.02	1.09	3.90

Wollemann, Montgomery and Foard (1951) report finding acid fast bacilli in tissue sections which were subsequently proved to be derived from the tissue mat used in preparing the sections. The bacilli had morphological and staining characteristics resembling those of *Myco. tuberculosis*. Prior to the discovery several erroneous diagnoses of tuberculosis had been made.

The organisms could not be grown in culture as the tissue mat must be heated to 56°C. to melt it, which apparently kills the organisms. The morphological and staining characteristics suggest that it may have been *Mycobacterium phlei*.

Experience with Non-Pathogenic Acid Fast Bacilli.

From two patients on two successive days stomach washings were received. They were from the same ward and presumably received similar treatment. The specimens were concentrated with caustic soda and films and cultures made. In all the films large clumps of rather filamentous acid fast bacilli were found, but as they were not typical of tubercle bacilli a note to that effect was put on the reports. Cultures were made but no growth was obtained. The specimens were not expected to be positive and the result was rather embarrassing, as both patients were nurses. No explanation could be given, as no similar results were got before or since. The staining was checked and in both cases the organisms were obviously in the deposit.

From the stomach washings of a Maori patient suffering from pulmonary tuberculosis colonies of an acid fast but not alcohol fast bacillus were isolated on Edson's medium. The colonies at this time were smooth and golden yellow and easily emulsified. It grew slightly on plain agar; on Edson's medium there was visible growth at room temperature in 2 days, and quite obvious in 5 days, at 37°C. more obvious growth. A guinea pig inoculated with a large amount of culture showed no lesion after six weeks. Dr. Edson reported that the organism was similar to one they isolated from a tuberculous gland. He mentioned that Webster (of Melbourne) had recorded such organisms from gastric contents. Our strain (and Dr. Edson's), he said, closely resembled *Myco. kedrowsky*.

Another chromogenic acid fast bacillus was isolated from stomach washings of a nurse. This grew at 37°C. on plain and blood agar in 3 days, on Loeffler's serum and in broth, but more slowly at room temperature. It was both acid and alcohol fast.

The colonies were smooth and easily emulsified and at first of an orange colour, but later a variant was obtained with a very profuse growth of a beautiful salmon colour. The original culture was also non-pathogenic to a guinea pig killed after 6 weeks, although the inoculation was heavy. No further tests were done, although a subculture was sent away.

A specimen of synovial fluid from a knee was received with practically no details. The fluid contained numerous polymorphs, but no bacteria were seen in a Gram stained film and the cultures were sterile after 24 hours. A Ziehl-Neelsen film was examined as a routine procedure and showed moderate numbers of acid fast bacilli rather longer and more filamentous than typical tubercle bacilli, and a note of this was put on the report. A culture was made on Loewenstein-Jensen medium. Nothing further was heard of the matter until about a fortnight later, when the Loewenstein culture was examined and showed large colonies of a pinkish buff colour not typical of tuberculosis, but sufficiently suspicious-looking to warrant investigation. They proved to be acid fast bacilli. The ward was informed and further details came to light. The organism was found to grow on all ordinary media, including MacConkey agar, at 37°C. within 2 days, and at room temperature in a number of days. Two guinea pigs inoculated (one with a heavy inoculum and one with a small inoculum) showed no lesion when killed at 6 weeks, although there was some swelling in the first week. Dr. Edson reported:—

Classification Class IIa 1 of Gordon. It appears that it is a soil organism.

When the report of the culture after a fortnight went to the ward ,the patient had been sent home, as nobody believed that he had a tuberculous infection of the knee and the condition cleared up. Further investigation showed that the man had punctured his knee with a hayknife and the synovitis had followed. He was able to work soon after going back to his farm, and showed no ill effects.

It is of interest to note a report from Adelaide appearing in the press on 18th August, 1951, quoted from the *Medical Journal of Australia*, that in Melbourne Children's Hospital acid fast bacilli from slime in water-taps had proved fatal to a rabbit and that the organisms were indistinguishable from tubercle bacilli.

My thanks are due to Dr. D. N. Allen, Pathologist, for his permission to publish these reports.

References:

Schwabacher, H. (1933). Spec. Rep. Ser. Med. Res. Coun. London, No. 182, Tuberculous Bacillaemia, Appendix C. P. 124.

Gordon (1937), Jour. Bact. 34, 617-630. Classification of Acid Fast Bacteria.

Gordon & Hagan (1939), Journ. Bact. 36, 39. Classification of Acid Fast Bacteria.

Wollemann, Montgomery & Foard (1951), Am. Jour. Clin. Path., Vol. 21, 3, 295.

GENERAL

Comparison of the Rat and Friedman Tests for Pregnancy. The results of this study suggest that the rat ovary hyperemia test is less sensitive than the Friedman test, but is more likely to give the correct negative result, as the more sensitive Friedman test may give false positive results in non-pregnant women in clinical conditions such as menopause which are associated with an increased titre of pituitary gonadotropic hormone in the urine.—Hoffman, R., Markey, R., and Giordano, A. (Amer. J. Clin. Path., Vol. 21), *33,* 1951.

A Holder for Test Tube Baskets. To minimise the tendency of tubes to fall over in the basket whilst tubing media, an easily made holder of great convenience is described. The holder is made with equilateral sides, so that the rectangular basket will assume a constant position. The only critical feature is that the depth of the holder be sufficient to keep the bottom corner of the basket off the bench. A holder with sides having an internal length of six inches and a depth of 2½ inches is suitable for holding baskets having a width of 4 to 6 inches.—Elsdon-Dew, R. (Amer. J. of Clin. Path., Vol. 21), *99,* 1951.

A Bone Bank. This article discusses the procurement, preparation and storage of homogenous bone for use as isografts in selected cases. Similar methods may be used for preparing and storing cartilage.—Gordon, H., and Welsh, B. (Amer. J. of Clin. Path., Vol. 21), *114,* 1951.

Detoxifying urine in Frog Test for Pregnancy. An easy method is presented for detoxifying human urine using the ion-exchange resin Amberlite XE-96, which absorbs the toxic substances without removing the gonadotropins. To detoxify a sample of urine, about 3 gm. of the prepared resin is added to about 10 ml. of urine in a test tube. The mixture is shaken vigorously for a few seconds and the resin is allowed to settle. The clear supernatant consists of the detoxified urine.—Levey, S., and Putnamn, E. (Amer. J. of Clin. Path., Vol. 21), *197,* 1951.

Determination of Uric Acid in Blood and Urine. The chief source of error in the majority of standard methods seems to be concerned with the boiling time. The author has produced a modification of the method of Dr. Wolfson, of the Michael Rees Hospital, Chicago, U.S.A., which eliminates the necessity of boiling and is sufficiently constant in its results to enable a standard curve to be prepared. The principle of the method is the proteins are precipitated from plasma or serum using Folin's tungstic acid method. The filtrate is treated with urea-cyanide and an arseno-phosphotungstate reagent at room temperature, when a blue colour develops. After a standard period of time the colour intensity is read in a photo-electric absorbtiometer and the results compared with a standard curve prepared from uric acid solutions of known concentration.—Bidmead, D. (J. of Clin. Path., Vol. 4), *366,* 1951.

ANALYSIS OF SEMEN

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Collection of Specimen

1. Direct into a clean, dry, wide-mouthed glass jar.
2. Condom specimens unsatisfactory with regard to motility if allowed to remain in condom for any length of time.
3. Optimal interval from previous coitus probably 1–3 days; if there is apparent infertility or sub-fertility, collect a further specimen after a larger interval.
4. Specimens to be received within three to five hours after emission. When difficulty is experienced in meeting these requirements, periods up to 24 hours are permitted. Disregarding motility, reasonable information can be given regarding other characteristics.

Examination of Wet Preparation

1. Express motility as percentage. The specimen is usually examined at room temperature. Activity is increased at body temperature. Non-motile spermatozoa may be reactivated by warming. Viability may be observed at time intervals up to 24-28 hours.
2. Observe for the presence of:—
   (a) Cells of spermatogenesis.
   (b) Epithelial cells.
   (c) Pus cells.
   (d) Crystals.
   (e) Red blood cells.
   (f) Bacteria.

Differentiation of cells of spermatogenesis and leucocytic origin is difficult in wet preparations and should be left to the stained preparation.

Other abnormal constituents may be observed, e.g., contaminants from the female or male genital tract, contaminants from mode of collection, starch granules from rubber sheath used in collection ,etc. Having examined the wet preparation, record volume and note viscosity. (Semen normally liquefies within 3–4 hours after ejaculation.)

Estimation of Density of Sperm. Population

Diluent:—

1% Chloramine T in normal saline. This ensures a homogeneous dilution owing to its mucolytic properties.

Technique:—

Using haemocytometer, count as used for red cell count. Dilution of semen is dependent on sperm density, dilutions of 1/40, 1/20, 1/10 and 1/5 being satisfactory for most specimens. If available use 1cc. of semen for dilution, as semen is not entirely homogeneous.

Note:

If dilution is 1/5, then $\frac{X}{4} \times 10^6$ = spermatozoa per cc.

" " " 1/10, " $\frac{X}{2} \times 10^6$ = " " "

" " " 1/20, " $X \times 10^6$ = " " "

" " " 1/40, " $2X \times 10^6$ = " " "

Where X = Number of spermatozoa per 1/5 sq. m.m.

Preparation of Stained Film

1. Prepare film as for an ordinary blood film.
2. Allow to dry.
3. Fix in methyl alcohol for 2 minutes. Fresh methyl alcohol is essential for good preparations.
4. Wash with water.
5. Stain in Mayer's acid haem. alum.
6. Rinse with water.
7. Blue if necessary.
8. Counterstain lightly in 1% alcoholic or aqueous eosin.
9. Differentiate with water or 50% alcohol if necessary.
10. Dry.
11. Mount if desired.
12. Examine.

Note.—Thickness of smear must be relative to sperm. population. Examine stained film and estimate the percentage of:—

(a) Normal forms.
(b) Immature forms.
(c) Degenerate forms.
(d) Teratological forms.

Note if there is any variation in size and shape of spermatozoa. Report degree of anisozoospermia or poikilozoospermia.

Description of Spermatozoa

1. *Normal Forms.*
   (a) Head: Oval or somewhat pyriform and in profile converges at anterior margin to a thin edge. Clear or lightly staining anterior half and darkly staining chromatin mass in posterior half.
   (b) Tail: About 10 times the length of the head.
   (c) Middle Portion: A cylindrical area somewhat thicker than tail.

2. *Immature Forms.*

   Classified abnormal because they have not reached the adult stage. They may be motile. Characterised by cytoplasmic masses attached to, or completely surrounding head, body or tail. Tail may be rudimentary.

3. *Degenerate Forms.*

   Characterised by loss of regularity and distinctness of outline, vacuolization and poor affinity for stains.

4. *Teratological Forms.*

   Characterised by abnormal distribution of nuclear material or by abnormal size or shape of mature spermatozoa. Head may be solid staining or chromatin in anterior half of head or may be multiple masses of chromatin. May exhibit bizarre conglomerations of multiple heads and tails. May have small remnants of cytoplasm, but if latter large in amount, should be classified as immature.

Apparent Azoospermia

1. Add an equal volume of Chloramine T to the semen.
2. Mix thoroughly.
3. Centrifugalize for 5 minutes at 1500-2000 r.p.m.
4. Examine wet and stained preparations for cells of spermatogenesis or spermatozoa.

Note.—Careful examination of stained preparation is essential as demonstration of spermatozoa is of diagnostic importance.

Normal Variations

Volume: 2-6cc. Mode of collection may grossly affect volume as received.

Motility: 50% should be motile 3–5 hours after emission. The nature of the container is important. Rubber may be spermicidal.

Density: 50×10^6 — 500×10^6 per cc.

Morphology: Depending on criteria accepted at least 60% should show normality.

Definition of Terms

1. Azoospermia: Complete absence of sperms.
2. Oligozoospermia: Sperms. scanty in number.
3. Necrozoospermia: Death of sperms. before emission.
4. Asthenozoospermia: Rapid dying off of spermatozoa after emission.
5. Euzoospermia: Absence of manifest spermatozoal defect.
6. Isozoospermia: High degree of uniformity of sperms.
7. Teratozoospermia: Frequent abnormal head forms.
8. Aspermia: Absence of emission.
9. Oligospermia: Very small ejaculation of normal semen.

Tests Supplementary to Semen Analysis

1. *Post-coital examination of cervical mucus* (Huhner's Test): Examine wet preparations of withdrawn mucus for presence of spermatozoa, motility of spermatozoa, migration of any of the spermatozoa, cells, etc.
2. *Mucus-Sperm, Invasion Test*
   The husband's spermatozoa are placed in opposition to the cervical mucus on a slide, overlaid by a cover slip, care being taken that the semen does not flow over the mucus. Note invasion and migratory power of spermatozoa after 1 hour and 2 hours on incubation of sealed slide preparation.

Acknowledgments:

I wish to sincerely thank Dr. E. Holland for permission to publish this article, and Mr. N. Frost, of the Crown Street Women's Hospital, for his helpful suggestions, particularly on the "Description of Spermatozoa."

The Specific Gravity on One Drop of Urine. The principle of the proposed method is as follows: One drop of urine is placed in a standard, small volume of an immiscible, inert liquid which has a specific gravity lower than the minimum value for urine. The drop will sink to the bottom of the liquid. A denser liquid is added from a microburette until a mixture is obtained in which the drop of urine floats in equilibrium. The volume of liquid added is used to determine the specific gravity from a graph. The reference liquid has a S.G. of 0.992 and is prepared by mixing 50 ml. benzene (S.G. 0.879) with 50 ml. monochlorobenzene (S.G. 1.107). One drop of urine is placed in 1 ml. of the reference liquid. Monochlorobenzene is added drop by drop, the tube is gently swirled to mix the liquids after each addition. The end-point is reached when the drop of urine no longer falls, but remains suspended in the liquid or rises very slowly when the tube is at rest. The volume of monochlorobenzene added is noted, and the S.G. of the urine is read from the calibration curve. The reference liquids are stable indefinitely, and the method is reliable within 0.002 unit of specific gravity over the range 1.006 to 1.047.—Bowler, R. (J. of Clin. Path., Vol. 4), *491*, 1951.

Estimation of Carbon Monoxide with the Volumetric Van Slyke Apparatus. The author describes a simple attachment to the volumetric Van Slyke apparatus, which can be made by any technician, and which will permit gases to be ejected from and returned quantitatively to the chamber. This enables the chamber and gas burette to be washed out in the course of an estimation, and dispenses with the exposure of gas-absorbing reagents to a vacuum; the latter do not, therefore, require to be gas free. The reported accuracy is to ± 1.0% CO saturation.—Nicholas, J. (J. of Clin. Path., Vol. 4), *439*, 1951.

ISOLATION OF INTESTINAL PATHOGENS

F. M. Rush-Munro

(Department of Pathology, Auckland Hospital)

The following is a summary of the intestinal pathogens isolated during 1951 in the Bacteriology Department of the Department of Pathology, Auckland Hospital.

For faeces, the routine followed was an initial plating on to MacConkey agar, plus enrichment in Selenite F medium followed by plating on to MacConkey agar.

For blood cultures, bile broth was used initially with plating on to MacConkey agar.

The antisera used were obtained from the Standards Laboratory for Serological Reagents, Colindale, London.

S. typhi

Total no. cases.	No. times isolated.	Isolations blood.	Isolations faeces.	Average isolations per case.
26	69	15	53	2.6

One isolation from gall bladder.

S. paratyphi A.

Total no. cases.	No. times isolated.	Isolations blood.	Isolations faeces.	Average isolations per case.
6	20	5	15	3.3

Salmonellae

Type.	No. of cases.	No. of times isolated.	Average isolations per case.
S. typhimurium	43	175	4.0
Isolations faeces		170	
,, blood		1	
,, perianal abscess		1	
,, colostomy wound		2	
,, cooked corned beef		1	
S. bovis morbificans	1	2	2
S. derby	1	1	1
S. orion	1	1	1

Shigellae

Type.	No. of cases.	No. of times isolated.	Average isolations per case.
Sh. flexner Z	5	10	2
,, ,, 88	5	7	1.4
,, sonnei	17	29	1.7

Total isolations	314
Total number of positive cases	105
Average isolations per case	3.0

The Salmonella orion was kindly identified for us by Mr. S. W. Josland, of the Wallaceville Animal Research Station.

DEPARTMENT OF HEALTH

Final Qualification for Hospital Bacteriologists for Certificate of Proficiency in Hospital Laboratory Practice.

Examiners: Professor D'Ath, Dr. Mercer and Dr. Pullar.

Medical School, Dunedin, February 25th-27th, 1952.

PAPER—February 25th, 9.30-12.30 a.m. (Three hours.)

1. Describe how you would isolate and identify the various members of the Neisseria group.
2. Discuss the principles underlying the estimation of "prothrombin time," and its use in calculating the prothrombin content of the blood as a percentage of normal.
3. Give an account of the methods used in the pasteurisation of milk. How is the efficiency of pasteurisation tested in the laboratory?
4. Explain briefly the meaning of the following terms:—
   (a) Coombs' test.
   (b) Polycythaemia.
   (c) Genotype.
5. What are the optimum conditions of storage for the following materials, and why?
   (a) Citrated blood for transfusion.
   (b) Kahn antigen.
   (c) Stock cultures of fungi.
   (d) Calf lymph.

Practical A

February 26th, 1952. Time, 9.30-12.30.

1. The swab provided is from an abdominal wound suffered by a shunter in a country railway yards yesterday. Carry out the necessary laboratory procedures in order to:—
   (a) give an immediate report;
   (b) give the utmost useful information within 24 hours.
   Tabulate the procedures used, with your reasons for using them and the results expected from their use, and state what further procedures might be used fully to identify the organisms.
2. The faeces is from a case of enteritis from which S. typhimurium was isolated. The patient has been on Chloromycetin and Streptomycin (to which the Salmonella was sensitive) for two weeks, but his condition has suddenly deteriorated.: Carry out the necessary laboratory procedures, state your reasons for using them, and state your reports on the specimen.
3. (a) On what principles does high-power dark-ground microscopy depend?
   (b) Why is it used for examination of T. pallidum?
   (c) List the equipment needed for this.

(d) Tabulate the procedure in setting up a dark-ground for T. pallidum.

(e) Set up a dark-ground with the apparatus and bacillary suspension provided.

4. Identify the specimens A–F.
A—Taenia ovum; B—Ascaris ovum; C—Trichuris ovum; D—Pubic louse; E—H. influenzae in C.S.F.; F—Cercaria from Lake Wanaka.

Practical B

February 26th, 1952. Time, 2.30-5.30 p.m.

1. Examine and report on the blood films provided.
2. Examine and report on the deposits in the specimens of urine provided.
3. You are provided with a litre bottle labelled to contain a 5% solution of dextrose. Estimate the actual percentage of dextrose present.
4. (a) What is this piece of apparatus; what is its composition and porosity? For what purpose is it used? How would you clean it?
(Sintered glass filter.)

(b) What are these and for what are they used?
(Spectroscope and capsule marked 116°F.)

(c) What is this and for what purpose is it used?
(OY² filter.)

(d) What are these substances used for?
Gum ghatti 2% W/V, Diethylene dioxide.)

(e) What is this reagent and what do you know of its use and production?
(Folin and Ciocalteau reagent.)

ORALS

Dr. Mercer:

Blood sugars, T.N.P.N., bacterial filters, the cleaning of intravenous bottles, the methods of estimating total proteins, Thorne's test, Rh typing, antibiotic testing, Friedman test and frog pregnancy tests, T.B. cultures, urobilinogen estimation, Van den Bergh, icterus index, pigments in faeces and urine.

Professor D'Ath:

Antiseptic and Rideal Walker testing, phenol co-efficient T.A.B., pH meters and standard buffers, molar solutions, buffer for Leishman, Na and K estimation, colloidal gold, liver function tests, blood sugars, gum ghatti, dioxane, Nessler solution, normal solutions, products of digestion in T.N.P.N., elastic stains, histological stains, decalcification of bone, iron haematoxylin stains.

Dr. Pullar:

Electronic ampoule filler, muffle furnace, ascaris, threadworm and its collection, ankylostomiasis, iron in tissue, Casoni tests, solution and T.N.P.N., chloride in C.S.F., urine and blood and indices, checks on autoclave sterilisation, use of centrifuges, Nessler eosinophilia plasma preparation, acid phosphatase, haematorcrits the reagents, determination and significance of level.

NOTES ON BLOOD COUNTING

Most methods employed to estimate the actual number of cells are open to serious physical errors regardless of personal technique, and the latter itself may induce considerable errors in blood counting.

From a consideration of these two factors, some departures have been made from the usual methods.

These were introduced in an endeavour to eliminate certain mechanical errors, but also chiefly to simplify the procedure so that relatively inexperienced new members on the staff could perform accurate counts within as short a time as possible without having to acquire dexterity which comes with long practice.

In general the usual diluting pipettes have been discarded. Larger amounts of blood employed with correspondingly larger volumes of diluting fluid—the larger volumes of blood and diluting fluid tend to reduce errors in measuring and filling.

It will be generally agreed that filling a white cell pipette precisely and accurately is not always achieved.

Red Cell Counts:

A haemoglobin pipette 0.02 cmm. is used to contain the blood and this is discharged into 4 ml. of diluting fluid in a Kahn tube. The pipette rinsed several times by sucking up and down. The tube is inverted a number of times to ensure careful mixing. A small pipette is used to transfer a drop to the counting chamber, the drop being expelled before sedimentation takes place in the pipette tip.

The same Pasteur pipette may be used for a number of counts provided a small amount is drawn up each time, the excess blown out after delivery of the drop. Then the contents of the next tube sucked up and down to wash the pipette out before withdrawing the sample.

The dilution is 1 in 201 and the count proceeded with in the usual manner. The difference in dilution is disregarded, as it is considered the error is greater using the diluting pipette and this figure is well below the "normal error."

The diluting fluid is delivered from a self-filling burette of narrow bore graduated at 4 ml. intervals. The delivery tip is fine, so that the burette is emptied slowly with time to drain. A two-way tap connects to a large stock bottle of diluent on a shelf above.

White Cell Counts:

Again a Haemoglobin pipette 0.02 cm. is used. This is discharged into a 3 x ⅜" tube containing 0.38 ml. of white cell diluting fluid. This is delivered from a self-filling micro-burette.

The same procedure as for red cell counts is then followed using a different pipette, marked to distinguish it. The bottom of the tube is tapped vigorously to ensure mixing and not inverted as in the red cell count.

With reference to the two methods of counting. The new methods were run in parallel with the diluting pipette method and no significant variations noticed.

However, when an inexperienced person performed the counts a considerable error occurred using the diluting pipettes, while results consistent with checked values were obtained in most of the counts using the new technique.

Thus one standard type of sampling pipette is used for all blood counts. Of course a separate pipette is used for each individual count, red or white.

It is necessary to check the calibration of some makes of pipettes with mercury. However, reliable brands appear to be consistent.

Summary:—Methods for blood sampling and diluting are described which, it is believed, simplify the technique and dispense with errors caused by inexperienced manipulation and allow more accurate counts to be performed by relatively inexperienced staff, without a long period of practice.

In view of the constant changing of staff, this is of considerable assistance.

—G. George, Rotorua.

TECHNICAL HINTS

D. H. Adamson

(Department of Pathology, Christchurch Hospital.)

(1) Sterile Pipettes

It is convenient to store sterile graduated and bulb pipettes, of up to 10 ml. volume, in glass tubing, 1 inch longer than the pipette, drawn out and sealed at one end. After inserting the pipette, one end is plugged with cotton wool and the whole hot-air sterilised. Cotton wool in the base of the tube is undesirable and is unnecessary, as movement is restricted by the plug.

(2) Blood Cultures

Rubber-lined, screw-capped bottles are satisfactory for incubating blood cultures. A hole ⅛-inch diameter is drilled eccentrically in the metal cap. Before autoclaving the bottle of blood culture medium, this hole is sealed with a short strip of adhesive plaster, one end of which is doubled back for easy removal and the cap screwed tightly down. When the blood for culture has been withdrawn, the plaster is lifted, the needle inserted into the bottle through the rubber lining of the cap, the appropriate quantity of blood introduced, and the plaster replaced. No flame is necessary in this technique, and contamination is almost unknown. The rubber lining may be rotated periodically so that the same point is not punctured repeatedly. (A similar, though we feel less satisfactory, method is described by T. J. Mackie and J. E. McCartney, 1948.)

(3) To Dry Coverglasses:

After washing, place in methylated spirits.

Place a single layer of soft linen cloth, 2ft. x 1ft. in size, on the bench.

Take about ½oz. of coverglasses out of spirit and scatter at the far end of the cloth, which is held down with the left hand.

With another cloth, folded into a smooth ball, briskly sweep the coverglasses towards you with the right hand.

Now sweep them back at the far end.

About six sweepings, occupying less than 3 minutes, will thoroughly dry ½oz. of coverglasses.

(4) To Dry Microscope Slides:

The method is similar to that for coverglasses above, except that a pile of about 10 slides, after removal from spirit, should be stacked with their longer edge facing you at the far end of the cloth and slowly drawn, one by one, towards you until they are in a single layer. To dry the upper surface, brush the cloth held in the right hand lengthwise across them, when the friction of the damp cloth on the bench will hold them in position. Half gross of slides can be polished in less than five minutes.

SELECTED ABSTRACTS

L. Reynolds

(Department of Pathology, Wellington Hospital)

Bacteriology and Parasitology

The behaviour "in vivo" of particulate antibiotics. Experiments were undertaken to investigate the behaviour in the animal body of an almost insoluble antibiotic and of its effect on experimental tuberculosis. It is removed from the circulation by phagocytic cells, in which it can be detected for months by fluorescence microscopy; it can also be estimated in tissue by its antibacterial activity. It is slowly eliminated, probably mainly in the bile. The results, to be reported later, as to its behaviour in experimental tuberculosis were disappointing, but indicate a worth-while method of investigating other insoluble antibiotics with perhaps greater power to inhibit the growth of the tubercle bacillus. Markham, Heathey, Sanders & Florey (Brit. J. of Exper. Path., Vol 32, *136,* 1951.

The envelope substance of Pasteurella pestis. Amies finds that the so-called envelope substance is simply a well-developed capsule, and can be dissolved in a solution of potassium thiocyanate. The antigen extractable from this solution appears to be a relatively simple protein, and possesses a high degree of immunizing efficiency. Amies, C. R. (Brit. J. of Exper. Path., Vol. 32), *259,* 1951.

Blood Media for the cultivation of M. tuberculosis. Many workers have previously shown that the incorporation of blood into the basal media supported the growth of a large inoculum of virulent human tubercle bacilli. This article shows that whenever blood was present in adequate concentrations (15–50 per cent.) the growth of small inocula of tubercle bacilli regularly occurred. This was shown to be true regardless of the basal medium used. The final blood medium selected was Bordet-Gengou agar or blood agar-base hydrated with 1 per cent. glycerine and containing 25 per cent. bank blood. Both the dehydrated basal media and the outdated bank blood are readily obtainable and small quantities of fresh media may be quickly prepared. Tarshis, M., and Fusch, A.W. (Amer. J. of Clin. Path., Vol. 21), *101,* 1951.

Sensitivity of Bacteria to Chloramphenicol in vitro. The relative susceptibility to the antibiotic was estimated by a paper disc method similar to that described by Bondi, the criteria of susceptibility being based on the usual levels of Chloramphenicol attained in peripheral blood. These levels vary between 5 and 12 mg. per ml. The method described was found to be simple, rapid, and of practical value for estimation of sensitivity to various antibiotics.—McLaurin, Tuttle and Beamer (Amer. J. of Clin. Path., Vol. 21), *189,* 1951.

A single medium for detecting Urease and Indole. A liquid medium is suggested which enables the *proteus* and *paracolon* groups of bacteria to be determined by their characteristic reactions in the same test tube. This is done by the use of a simple indicator, phenolphthalein, which is red in alkaline solution and colourless in acid, thus permitting indole to be tested by chemical means. Culture medium: Difco Tryptone, 1 gm. 0.9 per cent. saline, 100ml. 1 per cent. alcoholic solution of phenolphthalein, 0.5 ml. 50 per cent. urea solution, 1 drop. The tryptone peptone is dissolved in saline, the phenophthalein solution is added ,and the medium distributed in about 1ml. quantities in small test tubes and sterilised for 20 mins. at 115°C. Concentrated urea solution is prepared 50 gm. of urea dissolved in 100 ml. of distilled water. This solution becomes sterile after standing one day at room temperature, thus avoiding the tedious Seitz filtration. It should be stored in a previously sterilised dropping bottle. One drop of this solution is added to a tube of sterilised medium at time of inoculation with one non-lactose fermenting colony. Generally six hours is sufficient incubation to show the splitting of urea by reddening of the medium; also indole can be tested for by

adding the indole reagent which contains hydrochloric acid, which neutralises the ammonia if present, changing the medium from red to colourless. The amyl alcohol of the indole reagent carries the indole to the surface, where it appears as a bright red ring..—Rappaport, F. and Henig, E. (Amer. J. of Clin. Path., Vol. 21), *789,* 1951.

Treatment of Chronic Typhoid Carriers with Terramycin. Twelve chronic typhoid carriers treated with terramycin hydrochloride in a dosage of 1.25 gm. by mouth every six hours for ten days persisted in excreting *S. typhi* in their stools after completion of treatment.—Korns, R. & Albrecht, R. (J. of Lab. & Clin. Med., Vol. 38), *617,* 1951.

Comparison of In Vitro Sensitivity of Bacteria to Terramycin and other Antibiotics. 133 strains of bacteria, most of them isolated from hospital patients, were tested in vitro for sensitivity to terramycin, aureomycin, chloramphenicol, and streptomycin. Gram positive cocci were also tested with penicillin. The antibiotic spectrum of terramycin generally resembles that of aureomycin, although terramycin inhibited *Pseudomonas* strains in lesser concentrations and, like chloramphenicol, showed greater activity against *Shigella.* Three *Brucella suis* strains were highly sensitive to terramycin, aureomycin, streptomycin and chloramphenicol. When the organisms were recovered in relapse after treatment with terramycin, there was no increase in resistance to this drug. Four of fourteen strains of *Staph. aureus* and five of ten strains of *Staph. albus* were highly resistant to terramycin, requiring 200 or more mg. per ml. for inhibition. Some strains were more resistant to chloramphenicol than to the other agents. Striking variation was noted in sensitivity of *Pseudomonas and Enterococcus strains* to all antibiotics.—Ransmeier, Brown & Davis (J. of Lab. & Clin. Med., Vol. 38), *620,* 1951.

Serum Concentrations of Chloramphenicol. In fifty patients serum chloramphenicol levels were determined following the intravenous administration of a new parenteral preparation. Following a single injection of 1.0 gm. serum concentrations averaged 20 mg. per cc. at 1 hour and gradually declined to 2.4 mg. at 24 hours. When levels following oral and intravenous administration were compared it was found that the intravenous route was slightly higher at three hours, but was twice as high at twelve hours, and persisted for a longer period than with the oral route. The parenteral preparation was also well tolerated by the intramuscular route, but did not give serum levels adequate for the therapy of most infections.—Burnell and Kirby (J. of Lab. Clin. Med., Vol. 38), *234,* 1951.

Inhibition of Bacterial Growth by Adsorbed Anionic Detergents. In bacterimetric work using *Streptococcus faecalis,* great variations of growth response in parallel experiments was noted by the authors. These inconsistencies were traced to the method of cleaning the culture tubes. It was then demonstrated that the soap used left inhibiting residues of great persistence; next it was shown that the use of synthetic detergents could cause inhibitory effects which could not be reliably eliminated by twenty rinsings with cold distilled water. Thus glassware washed with soap or surface-active detergents is not fit for quantitative bacteriologic experiments because these cleaning agents become adsorbed on the glass surface and adhere there persistently. Methods of removal by oxidation and desorption autoclaving are presented.—Toennies, Gallant, Rahn and Ista (J. Lab. Clin. Med., Vol. 380), *163,* 1951.

Effect of Antibiotics on Human Faecal Flora. The practical value of reduction of the flora of the gastrointestinal tract as pre-operative preparation has been previously noted. The authors studied the effect of prolonged antibiotic administration and were able to demonstrate that: Aureomycin or Chloramphenicol, or both simultaneously, were effective in significantly suppressing the faecal flora for long periods. Penicillin and dihydrostreptomycin administered parenterally in combination were unable to materially influence the faecal flora. Aureomycin caused the prompt disappearance of coliform

organisms from the stool with marked reduction in the usual associated flora. Resistant organisms appeared as the effective antibiotics suppressed the normal coliform bacteria and enterococci, but the normal flora returned within 48 hours after the drugs were discontinued. As the coliform bacteria were suppressed, so were the faecal odour and pigment.—Bierman and Jawetz (J. Lab. Clin. Med., Vol 37), *394*, 1951.

Polymyxin B. is an antibiotic isolated from *Bacillus aerosporus* and can be utilised with satisfactory results in serious infections which do not respond to other measures. It is particularly effective against some of the gram negative pathogenic bacteria, infections caused by *E. coli, A. aerogenes, Ps. aeruginosa Shigella* and *H. influenzae*. Polymyxin is the most effective drug at this time against serious infections due to most strains of *Ps. aeruginosa*. —Kagan, Kreusky, Metzer and Locke (J. Lab. Clin. Med., Vol. 37), *402*, 1951.

The Disc Technique for Determining Antibiotic Sensitivity. The dried disc method is a simple, rapid, but crude method of determining sensitivity to the antibiotics. The technique must be standardised to minimise the inherent errors of the test. It is important to note that a uniform heavy growth is required and that the concentration of the antibiotic should be carefully controlled. The authors recommend the use of Standard discs sterilised by dry heat, impregnated with varying strengths of the antibiotics by adding to each disc one drop (0.033 ml.) of the following strengths: shapes to be used for the various antibiotics. The following amounts per disc are recommended: Penicillin 1.5 units, streptomycin 33 mg., aureomycin 10 mg., terramycin 10 mg., and chloramphenicol 33 mg. These are prepared by adding to each disc one drop (0.033 ml.) of the following strengths: penicillin 45 u/ml., streptomycin 1,000 mg./ml., aureomycin and terramycin 300 mg./ml., and chloramphenicol 1,000 mg./ml. The use of these discs can give valuable practical information of the sensitivity of an organism. In any doubtful case, more accurate methods of assay should be carried out.—Fairbrother, R. and Martyn, G. (J. Clin. Path., Vol. 4), *374*, 1951.

A Heat-labile Brucella-Agglutinin-Blocking Factor in Human Sera. Recent work on the detection of Rh. agglutinins has refocused attention on "non-agglutinating," "incomplete" or "blocking" antibodies in agglutination test sera. Part of this attention has been directed to bacterial agglutination tests. Eighteen of 498 sera giving negative results, Brucella agglutination tests in the fresh unheated state, showed the presence of Brucella agglutinins blocking at titres of 1:40 to 1:320. The blocking effect was completely eliminated by heating at 56°C. for 30 minutes. The developing test proved that the Brucella agglutinin blocking is due to an antigenic component of human serum, probably a non-agglutinating antibody.—Schuhardt, Woodfin and Knolle (J. of Bact., Vol. 61), *299*, 1951.

The Sensitivity of Bacteria to Neomycin. The initial report by Wakeman and Lechevalier (1949) of the discovery of the antibiotic, neomycin, indicated the over-all spectrum. However, the author has investigated the sensitivity to neomycin of 171 freshly isolated human pathogens. Neomycin is highly active against most strains of *A. aerogenes, K. pneumoniae*, and *Micrococcus aureus var. pyogenes*. With the exception of the clostridia, it was equally effective against many strains of gram positive rods. The streptococci and pneumococci exhibited varying degrees of sensitivity, in general tending to be rather resistant. Many *Proteus* and *Pseudomonas* strains were moderately sensitive but exhibited unusual potentials for the development of resistance to neomycin.—Clancy, C. (J. of Bact., Vol 61), *715*, 1951.

Filterable Forms of Bacteria. The object of this article is to review work done on the filterable forms of bacteria, particularly the L phase. The L phase was first discovered in *Streptobacillus moniliformis* and is regarded as a phase of bacteria which produces a large number of small filterable viable elements.—Kleineberger-Nobel, S. (Bact. Reviews, Vol 15), *103*, 1951.

Laryngeal Swabbing and Gastric Lavage. A comparison was made of these two methods of collecting specimens for culturing tubercle bacilli from the same patients. Laryngeal swabbing gave growth in 14.8 per cent. of 802 tests, and gastric lavage in 5.5 per cent. of 618 tests. In untreated sputum-negative patients the percentage of samples showing growth was 25.9 in laryngeal swabbing and 11.2 per cent. in gastric lavage. The author suggests that gastric lavage can be discontinued as a normal method and replaced by laryngeal swabbing.—Gifie, A. (Brit. Hygiena, Vol. 26), *476,* 1951.

Brucella Blood Cultures. Failure to cultivate brucellae from the blood of some cases may be due to inhibition of growth by antibodies present in the serum. The authors recommend that the blood be collected in citrate and centrifuged; the sedimented cells are lysed in distilled water and centrifuged. the final sediment is used for cultivation.—Pickett, M. and Nelson, E. (J. of Bact., Vol. 61), *229,* 1951.

Diagnostic Methods in Tuberculosis. This Symposium is prepared by a group of authors and consists of three sections. I: The microscopic examination of stained material. II: Demonstration of *M. tuberculosis* by culture. III: Animal Inoculation. Only a general summary of this excellent symposium is possible here, but reference to the original article should be made by all engaged in this work.

1. The extensive social and economic adjustments for the patient which usually follow a diagnosis of tuberculosis make it mandatory that such a diagnosis be made only after *M. tuberculosis* has been identified either in culture in competent hands or by producing the disease in guinea pigs.
2. Because a few cultures have equivocal diagnostic features, and because some strains culturally typical of *M. tuberculosis* are actually avirulent in guinea pigs, it is recommended that cultures be tested for virulence before a final diagnosis of tuberculosis is made.
3. Attempts to make a diagnosis of tuberculosis based on smears may be misleading. The smears may not disclose the presence of tubercle bacilli, while cultures or guinea pig inoculation may prove their presence, and smears from such materials as urine, sputum, gastric washings or exudates from sinuses may contain saprophytic acid-fast bacilli which cannot be distinguished morphologically from tubercle bacilli.
4. Animals inoculated with material for the diagnosis of tuberculosis should be examined with due care to prove that the lesions are tuberculous or to indicate some other diagnosis. If lesions are minimal or local it may be necessary to inoculate the material into a second set of animals to be certain of the diagnosis.
5. Repeated examination of exudates, of specimens of sputum, gastric juice or other material, may be necessary in order to establish a diagnosis of tuberculosis.—Weed, L. Smith, C. and Cummings, B. (Amer. J. Clin. Path., Vol. 21), *673,* 1951.

Non-specific inhibition by serum and urine in the microbiologic assay of antibiotics. Of sixty combinations of normal serums with bacteria under standard conditions, twenty-nine, or barely half, yielded growth at a 1: 12 or greater concentration of serum, while one-third were still negative at 1: 25 serum to broth ratio. A considerable number of drug-free urines prevented growth of two test organisms at dilutions with the usual bio-assay range. These results throw considerable doubt on the assay results attributed to antibiotics when normal or pooled body fluids are used as controls.—Gerstung, R. (J. Lab. Clin. Med., *37,* 575).

Rapid Methods for the Detection of Motility. When small tubes, containing 1 or 2 ml. of S I Medium (Difco) or Trypticase Agar Base (Semi-solid) (BBL), are preheated to 37°C., "stab-inoculated" from a young agar slant culture, and incubated a water bath at 37°C., motility may be observed in a fraction of the time required by the usual methods. Motility could usually be observed within 90–120 minutes. When tubes containing 1 ml. of nutrient

broth, are preheated to 37°C. and heavily inoculated from young agar slants or from colonies growing on agar plates, motility can be observed in hanging drop preparations within 15–30 minutes.—(J. of Bact., *62*, 347).

Stability of antibiotics for tube dilution method of determining antibiotic sensitivity. Antibiotics were dissolved in normal saline and in concentrations ranging from 250 mg./ml. to 100,000 mg./ml. Streptomycin, chloramphenicol, penicillin, terramycin, neomycin, Q-19 and Polymyxin B were stable when stored in concentrated solution at 4°C for as long as six weeks. Aureomycin retained its potency in concentrated solution for only 48 hours. Both dilute and concentrated solutions of the above-mentioned antibiotics remained stable in the frozen state for six months.—Warbren, Carr and Dunnette (Amer. J. Clin. Path., *21*, 884).

PATHOLOGY

The Hotchkiss-McManus stain for the diagnosis of Fungus Diseases. Fungi causing superficial and deep mycoses have been shown to be selectively stained in tissue with the Hotchkiss-McManus technic, taking various shades of red while the animal tissue remains unstained.—Klegman, Mercon and De Lamater (Amer. J. of Clin. Path., Vol. 21), *86*, 1951.

A modified Ziehl-Neelsen Method for demonstrating Leprosy Bacilli and other Acid-fast organisms. A new method has been elaborated to demonstrate leprosy bacilli in formalin-fixed paraffin—embedded tissue. This has been regarded as difficult to accomplish with the routine methods because leprosy bacilli are only weakly resistant to acid decolorisation. This stain may be extracted during the subsequent dehydration and clearing procedures. —Putt, F. A. (Amer. J. of Clin. Path., Vol. 21), *92*, 1951.

Coagulated Plasma as an Embedding Medium. A method is presented for studying cells for evidence of malignancy using coagulated plasma as an embedding medium. Sections made by this technique are easier to read than smears. Essentially, this consists of preparing a block for sectioning by concentrating a layer of cells between two layers of coagulated plasma. Such a procedure is advantageous in that much less time is needed to study the specimen and a stain is used which is familiar to all pathologists. This technique may be applied to vaginal secretions, ascitic and pleural fluids, urine and bronchial, prostatic and gastrointestinal secretions.—Carson, C. P., and Dapene, A. V. (Amer. J. of Clin. Path., Vol. 21), *96*, 1951.

Sponge Technique for Biopsy in detecting Cervical Squamous Cell Carcinoma. In 1948 Gladstone introduced a new method for obtaining a cytological specimen. In this procedure a gelatin or cellulose sponge was employed to absorb tissue juice, cells and small tissue fragments from the site in question. After the specimen was obtained the sponge was placed in 10 per cent. formalin and submitted to the laboratory, where it was embedded in paraffin, sectioned and stained with haematoxylin and eosin. The purpose of this article is to evaluate the accuracy of this technique as compared with those obtained by examination of "smears" or tissue spreads made from the cervical secretion and scraping of the cervical portis vaginalis. The study of 400 patients by both methods revealed 26 cases of proved cervical squamous cell carcinoma. Examination of tissue spreads was positive in all 26 cases and sponge sections permitted recognition of 23 cases.—Reagan, J. W. (Amer. J. Clin. Path., Vol. 21), *357*, 1951.

The use of Ion Exchange Resin in Decalcification of Bone. The decalcification of bone for cytologic study has never been quite satisfactory. Not only is the process slow, but also the cellular detail obtained usually leaves much to be desired. The method described would appear to be probably the most suitable as yet devised. A mixture of cation exchange resin and formic acid was found to have many advantages.

1. The cellular detail of tissues is well preserved and is far superior to that obtained by a sodium citrate-formic acid control method. Indeed, the detail compares favourably with that obtained in non-decalcified tissues.
2. The method is faster than most.
3. The daily changing of solutions is eliminated.
4. The resin can be reclaimed.

—Dotti, Paparo and Clark (Amer. J. of Clin. Path., Vol. 21), *475,* 1951.

The Aliochrome Procedure. This is a differential method segregating the connective tissues, Collagen, Reticulin and Basement Membranes, into two groups. The application in sequence of the periodic acid Schiff reaction and the picro-methyl blue collagen stain tends to discriminate sharply between certain tissue elements which are coloured selectively by either method when it is applied alone. Collagen fibers usually take the blue stain, glomerular and renal tubule basement membranes the red. Some fine reticular fibrils and more delicate basement membranes and the sarcolemmate appear to be intermediate and may vary from red to violet-black to blue. The procedure of sequence periodic acid, Schiff sulphite leucofuchsin picromethyl blue staining is designated as the allochrome method.—Lillie, R.D. (Amer. J. of Clin-Path., Vol. 21), *484,* 1951.

A note on Frozen Section Technique. The following is a modification of Lillie's procedure for agar embedding which produces excellent results.

1. Tissue is placed in a test tube containing about 5 ml. 10 per cent. formalin, and this is brought to a boil.
2. The formalin is immediately replaced by 2 per cent. sterile agar in water, previously heated to its melting point, and the agar containing the tissue boiled gently for one minute.
3. The agar is poured off and the agar-infiltrated tissue is frozen at once. Sections are cut as usual, preferably on a rotary freezing microtome. Unlike gelatin, which stains with the basic aniline dyes, the agar is not stained by haematoxylin and eosin.—Friedland, L. (Amer. J. of Clin. Path., Vol. 21), *797,* 1951.

"Floating solution" for mounting paraffin sections. A mixture of egg white and glycerine is generally employed for affixing histologic sections to a glass slide. Uneven thickness of this mixture on the slide tends to cause undesirable staining artefacts by absorption of haematoxylin or eosin. The following modification is suggested. The egg white is separated from the yolk and filtered through two thicknesses of gauze, yielding a liquid volume of about 10 ml. The following stock solution is made up. Liquid egg white 10 ml., Glycerine 10 ml., Normal Saline to 1,000 ml., add few thymol crystals. The solution is stable but should be refrigerated.—Schleicher, E. (Amer. J. Clin. Path., *21,* 900).

HAEMATOLOGY AND SEROLOGY

An unusual Rh genotype. The red cells of a woman showing the Rh genotype —D—/—D— is described. It is advanced as the most likely explanation that the C and E genes have been involved in a small deletion. The serum contained anti-e, anti-C and anti-c Rh antibodies, presumably a result of immunization by pregnancy.—Race, R. R. Sanger, Ruth and Selwyn, J. G. (Brit. J. of Exper. Path., Vol 32), *124,* 1951.

Factors influencing the agglutinability of Red cells. Sensitized bovine red cells which did not agglutinate when treated with the appropriate anti-globulin sera were studied. Agglutination was produced after repeated alternate treatments with anti-globulin serum and globulin.—Coombs, Gleeson-White and Hall (Brit. J. of Exper. Path., Vol. 32), *195,* 1951.

The action of cholinesterase. Rabbits were immunized with a purified cholesterinase from ox erythrocyte stroma. Their serum inhibited the action of this enzyme. It also lysed ox red cells, and it is suggested that cholesterinase may be one of the antigens in these cells which stimulate haemolysin production.—Holland, W. C. (Brit. J. of Exper. Path., Vol. 32), *382,* 1951.

Heterophile antibody in Virus Hepatitis. Sera from 200 apparently normal adults and 65 patients with acute virus hepatitis were examined for their sheep-cell agglutination titres by the method of Davidsohn. The highest titre found among the normal adults was 1 : 56. One-fifth of the patients with hepatitis had a titre above 1 : 56, the maximum being 1 : 448 in one patient. Absorptions with guinea-pig kidney, beef red-cell and human liver antigens were performed in 13 patients with hepatitis with elevated titres. The results showed that the pattern of absorption of the heterophile antibodies should in most instances serve to distinguish virus hepatitis from infectious mononucleosis.—Leibowitz, S. (Amer. J. of Clin. Path., Vol. 21), *201,* 1951.

The Antiglobulin Test. This article is a complete survey of the technique and practical application of the identification of the various human isoagglutinins responsible for haemolytic disease of the newborn and haemolytic reactions to blood transfusions. The antiglobulin has been outlined in detail to show the advantages of its use in detecting antibodies specific for human erythrocytes but which fail to cause agglutination in saline. The preparation and standardization of the reagent is given and its application in Rh work is presented and the occurrence of false negative results is discussed.—Rosenfield, Vogel and Rosenthal (Amer. J. of Clin. Path., Vol. 21), *301,* 1951.

Mass Blood Grouping and Rh Typing. This very excellent article details the techniques of mass blood typing that may be applied to either small or large communities, making it possible to type large numbers of people in a relatively short time with maximum accuracy.—Davidsohn, Stern and Kashwagi (Amer. J. of Clin. Path., Vol. 21), *375,* 1951.

Rh Sensitization following transfusions of Rh-positive Blood into Rh-Negative Recipients. The incidence of sensitization to the Rh D factor in a group of 23 Rh negative male patients transfused with Rh positive blood was found to be 70%. A study of the case histories of these patients who received multiple transfusions revealed no single instance of severe haemolytic transfusion reaction. The 17 patients studied received a total of 184 pints of Rh positive blood (4 patients received 17, 24, 24 and 29 pints, respectively) and the highest resulting anti- Rh titre was 1 : 2048. Possible natural mechanisms of protection against multiple transfusions of Rh-incompatible blood are discussed. Although fear of the Rh factor as a cause of haemolytic transfusion reaction is perhaps exaggerated, statistical consideration affords no excuse for disregarding the Rh type in any individual case.—Klein, Konwaler, and Mahony (Amer. J. of Clin. Path., Vol. 21), *399,* 1951.

A Gravimetric method for the calibration of Haemoglobin Micropipettes. The inaccurate calibration of haemoglobin pipettes constitutes an important source of error in haemoglobinometry. The standard gravimetric method employing mercury lacks its usual precision when applied to haemoglobin micro-pipettes (20cc. mm.) because of the extreme difficulty in pipetting and manipulating minute quantities of mercury. A method is presented employing only the following apparatus: Tuberculin syringe; one-hole rubber stopper, size 0; petrolatum or stopcock grease; beaker; 50 or 100 ml. mercury; retort stand and clamp. The mercury and the pipettes are stored in the same location so that both will have attained the same temperature at time of calibration. The tip of the tuberculin syringe, the barrel of which has been heavily lubricated with petrolatum or stopcock grease, is fitted tightly into one end of the rubber stopper. The stopper is clamped to a heavy retort stand so that it is fixed firmly approximately 18 inches above desk level. The base of the pipette to be calibrated is inserted as far as possible into the other end of the stopper, and the whole assembly is adjusted to a vertical position. The arrangement provides a means of accurately controlling the column of mercury in the pipette and can be used with a degree of accuracy comparable with standard methods.—Stevenson, Smetters and Cooper (Amer. J. of Clin. Path., Vol. 21), *489,* 1951.

The clinical significance of increased rouleaux formation in smears of the peripheral blood. The author found that in 414 instances of excessive rouleaux formation 93 per cent. had significant organic disease. The sedimentation rate was roughly proportional to the degree of rouleaux formation, but protein changes were less consistent. The simultaneous occurrence of appreciable myeloid immaturity and excessive rouleax formation presumed evidence of underlying neoplasm but against leukaemia. When macrocytosis was noted with high-grade rouleaux formation hepatic disease was present. —Bayrd, S. (Amer. J. of Clin. Path., Vol. 21), *777,* 1951.

A Method of Detecting Rh Antibodies in Extremely Low Titre. Since the discovery of the Rh factors, a number of methods have been developed for the detection of antibodies specific for these antigens. Saline suspended cells are used for the detection of bivalent antibodies, and cells suspended in protein media as well as the antihuman globulin test (Coombs test) and trypsinated cell method have been used to detect univalent antibodies. The sensitivity of these vary, but the trypsinated cell method and the antihuman globulin test are the most delicate and yield the highest titres. The author of this article decided to apply the antihuman globulin test making use of cells previously treated with trypsin to compare with the previously mentioned standard methods. The results indicate that his method is of value to detect antibodies present in such low concentration that they are below the threshold of other methods, and it is suggested it may prove of value in identifying new antibodies.—Unger, L. J. Lab. & Clin. Med., Vol. 37), *825,* 1951. This method has already been used to demonstrate the Duffy Factor.

The L.E. cell. Lupus erythematosus cells are cells containing a homogeneous mass of apparently phagocytosed material which stains purple with Romanowsky dyes. These cells are found in blood and marrow treated with anticoagulants and never in fresh films. Lupus erythematosus cells are found very frequently in the bone marrow and peripheral blood of patients with acute disseminated lupus erythematosus, but only very exceptionally in other diseases.—Holman, S. (J. of Clin. Path., Vol. 4), *290,* 1951.

Inactivation of Wassermann Sera at 60° C. for 3 minutes.—The author divided 1,067 sera from a clinic into two parts. One part was heated at 56°C. for 30 minutes and the other at 60°C. for 3 minutes. When the sera were separated into syphilitic and non-syphilitic groups, more doubtful results were obtained with syphilitic sera heated at 56°C. than at 60°C. Less positive results were obtained on non-syphilitic sera heated at 60°C. than 56°C.—Velaudapillae, T. (B. of Hygiene, Vol. 26, No. 1, 39).

Diluting Fluid for the Eosinophil Count. (Thorn Test.) Eosinophil counts are widely used in the control of treatment by A.C.T.H. and adrenal steroids. The diluting fluid described here has several practical advantages over the Dungar and Randolf solutions and fulfils the main requirements of a diluent for this count. Formula for diluting fluid: Urea 50 gm., Trisodium citrate 0.6 g. Distilled water to 100 ml. Aqueous phloxine 2%, 5 ml. The fluid is prepared and centrifuged, the upper 100 ml. of the supernatant liquid is carefully pipetted into a clear receptacle. The remaining 5 ml. is discarded. The diluent keeps well at room temperature. It evaporates slowly and mixes easily. It stains cosinophiles pink and does not lyse them. It lyses other leucocytes and erythrocytes.—Manner, T. (Brit. Med. J., June *23,* 1951).

CHEMICAL PATHOLOGY

Photometric determination of Total and Free Cholesterol and the Cholesterol Ester Ratio of Serum. This modified Liebermann-Burchard reaction has been applied in the determination of known mixtures with an experimental error ± 5 per cent. The total and free cholesterol values of 25 normal adult sera and the cholesterol ester ratio was found to average 76.9 per cent. with a deviation of ±1.5 per cent.—Saifer, A. (Amer. J. of Clin. Path., Vol. 21), *24,* 1951.

The Quantitative Estimation of Calcium in human plasma by flame spectrophotometry. This deals with the use of the Beckman spectrophotometer Model DU, and Beckman flame photometer attachment. The procedure involves treatment with nitric-perchloric acid mixture and evaporation to dryness to destroy plasma protein and to free bound calcium. Finally the prepared sample and the calcium standard are determined with these optimal settings: Air, 20lb. per sq. inch. Oxygen, 30 inches of water, Gas (natural) 3 cm. Slit width, 0.2 mm. Wave length, 554 m. Cell, blue.—Mosher, Itans, Boyle, Myers and Iseri (Amer. J. Cl. P., Vol. 21), *75,* 1951.

The use of the Van Slyke Volumetric apparatus. The authors believe that the vast majority of laboratories' sole use for this apparatus is the determination of plasma carbon dioxide combining power. Thus the pathologist and the clinician are deprived of several determinations of considerable technical and clinical value. The authors suggest the apparatus should be used for:—

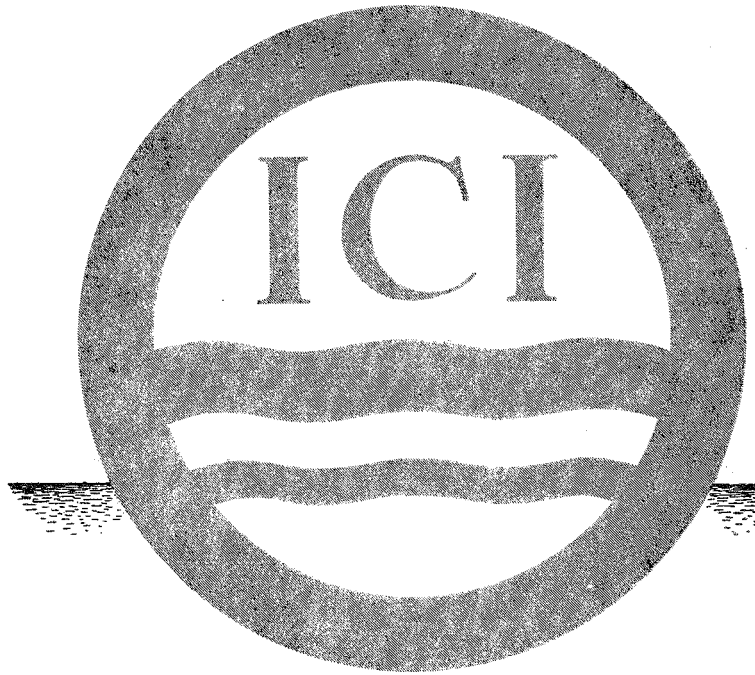
1. Whole blood oxygen capacity determinations, for the calibration of haemoglobinometers.
2. Arterial oxygen content, capacity and saturation determinations.
3. Whole blood and plasma carbon dioxide content determination.
4. Accurate blood carbon monoxide levels.

The standard techniques are reviewed with emphasis on certain details necessary for accuracy.—Lockhead, H. B., and Purcell, M. K. (Amer. J. of Clin. Path., Vol. 21), *177,* 1951.

Evaluation of Benzoyl Glucuronate Excretion Test (Snapper) for Liver Dysfunction. This test is based on the urinary excretion of conjugated glucuronic acid following the ingestion of a fixed quantity of benzoic acid. The test was found to have low sensitivity as a test of liver dysfunction, especially in cirrhosis, but gave satisfactory results in viral hepatitis. There seems little likelihood that the Snapper test will prove of value in distinguishing obstructive from non-obstructive jaundice. Employment of a series of liver tests in routine use, including icterus index or serum bilirubin, serum alkaline phosphatase, total protein and albuminin-globulin fractionation and the cephalin-cholesterol flocculation tests, gave more consistent diagnostic acid, especially in cirrhosis, viral hepatitis and obstructive jaundice.—Sharnoff, J., Badnick, M., and Jakab, G. (Amer. J. of Clin. Path., Vol 21), *234,* 1951.

Urea in Biologic Fluids. A method is described for the rapid estimation of urea in blood and urine using an Acid Diacetyl Reagent. To a tungstic acid filtrate of blood or serum, an equal volume of Acid Diacetyl Reagent is added. The mixture is heated for ten minutes, cooled and read in a Klett-Summerson colorimeter with a 44 filter. The colour produced is light-sensitive and the reaction is carried out in the absence of direct light. The error in this method is less than 3 per cent.—Natelson, Scott and Beffa (Amer. J. of Clin. Path., Vol. 21), *275,* 1951.

The Microdetermination of Chlorides in Serum and Spinal Fluids. This paper describes a rapid technique which is precise, accurate, and does not involve specialised reagents or unusual manipulation. Essentially, the procedure involves an argentimetric titration of an aliquot of the Somogyi (1945) filtrate using dichlorofluorescein as absorption indicator. The procedure is accurate to ± 2 per cent.—Franco, V., and Klein, B. (J. Lab. Clin. Med., Vol. 37), *960,* 1951.



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