remove a large—I believe the largest—group, which is easily recognized by its well-defined clinical features. It may be claimed with justice to have effected a veritable clearance in a lumber-room of diagnostic confusion. Most of us can recall the days when the chronic abdominal pains which are the subject of this study were ascribed to conditions as unsatisfying to the conscientious clinician as they were ill founded in pathology—for example, varicocele of the pampiniform plexus, sclerotic ovaries, ovaritis, prolapse of the ovary, constipation, adhesions, neurasthenia, etc.

The absence of gross pelvic and abdominal pathology and the puerperal correlation usually suffice at once to distinguish the syndrome from the major causes of lower abdominal pain—neoplasm, endometrioma, diverticulosis, appendicitis, etc. In subacute and chronic salpingitis there may be confusion, especially where the history dates from childbirth or abortion, but the presence of a tender, fixed, often bilateral mass in the posterior pelvis generally establishes the diagnosis. In some few cases the syndrome may be simulated by endometrioma; but here the puerperal correlation is usually absent, and whereas the former is very common in young women endometrioma is more frequent in later years. In endometrioma, moreover, the characteristic tender and fixed mass in the pouch of Douglas is often found. At the same time in women over 30 with a menstrual exacerbation of the pain endometrioma should always be considered. A chronic pelvic appendicitis may sometimes closely simulate the condition, though here again the characteristic puerperal dating is generally absent. It should be remembered that in such conditions as salpingitis, endometrioma, and pelvic appendicitis, where a tender swelling is present low in the pelvis, vaginal excitation pains are usually easily elicited, and therefore they cannot of themselves be regarded as of great value in the relatively rare cases which are in doubt. A common source of clinical confusion is in regard to recurrent appendicitis, and it is notorious that a large number of women with right iliac fossa pain have had their appendix removed before coming to our clinic. It is of the utmost importance that the surgeon should be more familiar with this common source of iliac fossa pain and its diagnosis. The easy establishment of referred excitation pain in the right side and its immediate removal by nerve blockage are the main diagnostic aids.

Finally, it is important to remember that in a large number of gynaecological conditions a serious error in diagnosis may arise from a failure to recognize that iliac fossa pain may spring not from the more apparent pathology, such as retroflexion, genital prolapse, etc., but from the frequently coincident cervical lesion. The institution of treatment appropriate to this lesion may eliminate the indication for more major treatment; where major measures are required the associated syndrome should be dealt with at the same time.

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CULTURAL METHODS IN BACTERIOLOGICAL DIAGNOSIS OF TUBERCULOSIS

BY

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Using the Jensen (1932) modification of the medium introduced by Löwenstein (1930), Holmes (1934) drew attention to the value of cultivation in the diagnosis of tuberculosis. Of the many observations of similar nature may be mentioned those of Hohn (1926), Sweany and Evanoff (1928), Bezançon and Buc (1931), Herrold (1931), Corper (1932), Danbolt (1935), Cummins and Duggan (1934), and Mishulow and others (1934). Shrewsbury and Barson (1937) noted successful results following the introduction of cultivation as part of the routine investigation of selected specimens of sputa. In order to determine the practical value of the method the same procedure has been adopted in this laboratory with all specimens in which an examination for *B. tuberculosis* was requested, and the results obtained over a period of two years are here recorded. Sputa from recognized or clinically suspicious cases of human tuberculosis comprised the bulk of the material examined, which also included specimens of urine, pleural fluid, milk, and cerebrospinal fluid. The previous routine examination was adhered to and the results compared with those secured by the additional aid of cultivation.

Methods

Routine Examination.—The examination of sputa consisted in the usual microscopical examination of films stained by the Ziehl-Neelsen method. The finding of acid-fast and alcohol-fast bacilli morphologically identical with *B. tuberculosis* was reported as a "positive" result, the absence of such forms being reported as "tubercle bacilli not found." The same procedure, preceded if necessary by centrifugation, was adopted with all other specimens save in the case of milk. Guinea-pig inoculation was carried out in specimens other than milk only if special request was made, but in examining milks the animal test was invariably utilized.

Cultural Methods.—If the specimen received was likely to be free of organisms other than tubercle bacilli—for example, cerebrospinal fluid withdrawn at lumbar puncture—the centrifuged deposit of such specimens was directly inoculated on culture media without further treatment. In the presence of contaminating organisms the material was first centrifuged if necessary. The deposit was then suspended in 2 c.cm. of the supernatant fluid, acidified for thirty minutes by the addition of an equal volume of 6 per cent. hydrochloric acid, and neutralized by the addition of 4 per cent. sodium hydroxide. The centrifuged deposit was then inoculated by means of a sterile Pasteur pipette on culture media. In a previous examination of a large number of cerebrospinal fluids and specimens of pus for tubercle bacilli the preliminary acid treatment had been successfully used, and the same procedure was therefore adapted to the examination of sputum instead of the usual alkali method.

The culture media most frequently used were the Löwenstein-Jensen medium and a modification of the same in which the glycerin content was omitted. All specimens were cultured on both media in screw-cap bottles. Considerable economy in incubator space and the easy handling of large numbers of bottle cultures were secured by the use of a rack consisting of a wooden

board, one inch in thickness, drilled with holes just large enough to hold the base of a bottle and fitted with a plywood base. In a rack eight inches by twelve inches were carried forty-eight cultures, the flat surface presented by the tops of the bottles enabling racks to be piled one above the other, while still permitting free passage of air and uniform heating of the cultures. Incubation was continued at 37° C. for four weeks, and all cultures were then examined. Films of suspect cultures were stained by the Ziehl-Neelsen method, and on finding the morphologically typical acid-fast bacilli the pathogenicity of the strain was in many cases confirmed by animal inoculation. The expense involved prohibited the examination of all strains by this method. Random selection of cultures for the test was therefore made, but all strains which showed the slightest variation in cultural characteristics were included in the selection. Cultures from specimens of sputum were tested for virulence in forty-nine (34.5 per cent.) of 142 cases which had been reported as negative in the routine microscopical examination, and in sixty (15 per cent.) of 401 cases in which the direct film report was positive. The pathogenicity of positive cultures from specimens of pleural fluid, pus, urine, cerebrospinal fluid, and milk was thus confirmed. In no instance was a diagnosis based on the morphological and cultural characters of a strain proved erroneous on animal inoculation.

Results

Sputum.—As previously stated, the bulk of the material examined consisted of sputa from tuberculosis dispensaries and those received from practitioners for general bacteriological examination. In Table I are collected the results

TABLE I.—Results of Examination by Combined Direct Film and Cultural Methods of 3,306 Specimens for *B. tuberculosis*

Result		Specimen							Total
Film	Culture	Sputum	Pleural Fluid	Pus	Urine	C.S.F.	Milk	Faeces	
+	+	401	0	4	5	1	2	3	416 (12.5%)
—	+	142	3	5	7	3	5	0	165 (5.0%)
+	—	9	0	4	0	0	2	0	15 (0.4%)
—	—	2,244	57	85	212	19	74	19	2,710 (82.1%)
		2,796	60	98	224	23	83	22	3,306 (100%)

obtained by the combined method. Of 2,796 such specimens 552 (19.7 per cent.) were reported positive by direct film, cultural, or combined examinations. Cultivation alone was successful in 543, or 98.3 per cent. of all positive results obtained. In the remaining nine specimens re-examination confirmed the presence of tubercle bacilli in direct films, but always in scanty numbers. The most significant finding was that only 410, or 74.3 per cent. of all positive results, were detected by the routine film examination. Repeated examination by separate observers confirmed the absence of tubercle bacilli in the original stained smears of the 25.7 per cent. of sputa later proved positive by culture. In such specimens the colonies appearing were often few in number, but not invariably so. Indeed, the rich growth which sometimes occurred was surprising in the face of a negative film report. As indicated in Table II, only five sputa were subjected to guinea-pig inoculation; all were negative in the triple test.

Pleural Fluid.—No specimen of pleural fluid was found positive by direct microscopical examination, but three were detected by culture. In these the number of colonies appearing on the Löwenstein medium were one, three,

TABLE II.—Results of Examinations for *B. tuberculosis* in which Guinea-pig Inoculation and Cultivation were both Utilized

Specimen	Number	Cultural and Animal Results				
		C+ A+	C- A-	C+ A-	C- A+	C+ Ad
Sputum ..	5	—	5	—	—	—
Urine ..	17	3	12	1	1	—
Milk ..	77	4	61	3	9	—
Pleural fluid ..	8	1	6	—	1	—
Pus ..	4	1	2	—	—	1
C.S.F. ..	3	1	1	—	1	—
Gastric lavage	4	2	—	—	1	1
Totals ..	118	12	87	4	13	2

C = culture; A = animal inoculation; + = positive; — = negative; d = inoculated animal died before reading possible.

and four respectively, indicating the extreme paucity of the numbers of organisms present. Guinea-pig inoculation succeeded in one instance where direct film and cultural examination failed.

Pus.—Of the thirteen positive specimens of pus, mainly from lymphatic glands, direct film and cultivation failed in five and four respectively. As three of the negative cultural results were from old-standing lesions, the organisms seen in direct films were possibly degenerated or actually dead.

Urine.—Since this laboratory serves as a group laboratory for several municipal hospitals a large number of urines were received for general bacteriological examination. Animal inoculation being reserved for special cases, routine cultivation proved a useful accessory measure, seven of the twelve positive cases being detected by this method. One specimen negative by both film and culture was positive on animal inoculation. Another specimen in which very few organisms were seen in direct films was positive on culture but negative on animal inoculation. In view of this surprising result the pathogenicity of the culture was confirmed by animal inoculation.

Cerebrospinal Fluid.—In the three specimens positive on cultivation only, the colonies appearing were in each instance few in number. Cultivation failed in one specimen positive by the animal test.

Milk.—The preculture treatment outlined above was not successful in eliminating all acid-fast saprophytes such as the timothy grass bacillus. These strains appeared rapidly on culture media, being clearly visible within two or three days, and were thus easily differentiated from strains of bovine tubercle bacilli, which were not apparent for an interval of eighteen days or more. Further, the growths of saprophytic strains were luxuriant as compared with the sparse dysgonic growth of the pathogen. Nine specimens positive on animal inoculation were negative on culture, while three positive cultural results were negative on animal inoculation. The pathogenicity of these latter strains was confirmed by animal inoculation.

Correlation of Bacteriological with Clinical Diagnosis

Although in most cases in human subjects from which specimens had been received there was some clinical justification for the request that an examination for *B. tuberculosis* be carried out, the great increase in positive results obtained by the cultural method made it essential to discover to what extent the bacteriological diagnosis agreed with the results of other methods of examination.

Accordingly a questionnaire was prepared and sent to clinicians in charge of those cases in which a negative direct film examination was followed by a positive cultural report. Of the fifty-five replies received the bacteriological findings were confirmed in fifty instances by clinical examination, x rays, or other means. In twenty-nine cases the clinician was reasonably certain of his diagnosis, but the cultural report served to place the matter beyond all doubt. Illustrative of this type of case are the following.

Case 1.—This man, aged 55, was suspected clinically of having pulmonary tuberculosis of the fibroid type, but repeated tests by direct film examination were invariably negative. The clinician observed that "the positive result in culture revealed an active condition which previously had only been suspected."

Case 2.—A woman aged 22, in whom the clinical diagnosis, supported by x-ray examination, indicated pulmonary tuberculosis involving principally the left upper lobe. Direct film examination was negative, but positive culture confirmed the diagnosis.

Case 3.—This patient, a woman aged 56, was diagnosed clinically as having chronic bronchitis. The clinician reported that "the physical signs were always those of chronic bronchitis and obscured any underlying tuberculous findings . . . the positive cultural result was followed by further investigations in hospital," and these confirmed the diagnosis.

Case 4.—In this patient, a man aged 22, an extensive pleural effusion developed on the left side eight months prior to examination of sputum. Repeated examinations of the fluid were negative, but coughing persisted. Direct film examinations of the sputum were negative, but on culture a few colonies of *B. tuberculosis* appeared.

In the next series of twenty cases the bacteriological findings were relatively of much greater importance in that the clinical suspicion of tuberculous infection was slight and the specimen was submitted more for the purpose of exclusion than in the hope of obtaining a positive result. The following are cited as examples of this type.

Case 5.—This patient, a man aged 60, had had a slight cough for years which ceased when cigarette-smoking was curtailed, and was thought to arise from that source. He had a bad family history of tuberculosis, but has always enjoyed fairly good general health. The positive cultural report led to x-ray examination, which revealed uniform marked opacity above the right apex with deposits in the left infraclavicular region from the first to the third rib. The only abnormality detected clinically in the chest was a diminution in breath sounds over the upper part of the left lung.

Case 6.—A man aged 39; he was considered to have entirely recovered from pulmonary tuberculosis, and had returned to work as a shop assistant. His doctor advised him to report periodically for examination, and a specimen of sputum taken at one of these visits was negative by direct examination but positive on culture. Physical examination revealed no signs of active infection.

Case 7.—A married woman aged 41. She complained of a feeling of weakness and loss of energy; physical examination was entirely negative. X-ray examination, following the receipt of a positive cultural report, revealed a right basal pleurisy and light fibrotic disease of right apex. Sanatorium treatment for six months, concluded with a further x-ray examination, which showed that the pleurisy had cleared up and the right apical lesion had apparently healed.

Among this latter group of cases were several in which the infection was almost certainly of very recent standing.

Case 8.—A typist, aged 30. This patient had had a slight haemoptysis, but otherwise she felt well. Clinically there was nothing further suggestive of active infection, but x-ray examination on the receipt of a positive cultural report indicated a lesion in the left apex. After sanatorium treatment the patient recovered satisfactorily and returned to work.

Case 9.—A male, aged 32, employed as an able seaman, complained of haemoptysis. Clinically he appeared fit and was able to carry out his duties without difficulty. X-ray examination following a cultural report indicated a lesion in the left apex. In this instance the diagnosis was of importance as regards pension adjustments, etc., but an observation period in a sanatorium fully confirmed the diagnosis.

Case 10.—A baby boy, aged 11 months, had attacks of mild pyrexia at intervals over three weeks, which were followed by signs of consolidation at the left apex. The mother was known to be tuberculous, the family history being altogether bad. The patient was found to react positively to the Mantoux test, the concentration of tuberculin used being 1 in 100,000. In this instance the culture was of the human type.

In all the above cases the cultures were derived from sputa, but the organism has also been unexpectedly demonstrated in other situations by this method.

Case 11.—A boy aged 13 complained of prepatellar bursitis of left knee. This condition had persisted for months, to a great extent varying with his general health. On account of a bad family history, a specimen of pus aspirated from the lesion was submitted for examination. No growth appeared on blood agar and no organisms were found on direct films, but tubercle bacilli were isolated on culture.

CASES WITH LESIONS OTHER THAN TUBERCULOUS

In the remaining six cases there were lesions other than those of tuberculous infection to which the patients' symptoms were attributed by the clinician in charge. The possibility that tuberculous infection was also present, however, could not be entirely discounted in any of these cases, the details being as follows.

Case 12.—A married woman aged 39 complained of frequent head colds, with subsequent irritable cough, for four years, dating from childbirth. She was ambulant, but always appeared unwell. Clinically and radiologically there was no evidence of tuberculous infection, but tubercle bacilli were isolated on culture from sputum.

Case 13.—A woman aged 40 was admitted to hospital with "meningitis of doubtful origin," later considered to be cerebrospinal meningitis on clinical grounds and on the isolation of meningococci from cerebrospinal fluid in the hospital laboratory. A specimen of fluid submitted to this laboratory for verification was found to show no organisms in direct films and no growth on blood agar, but tubercle bacilli were isolated on culture. The patient recovered without further developments and was discharged apparently well.

Case 14.—A man aged 63 was admitted to hospital as a case of congestive heart failure. Clinically there was no evidence of tuberculosis, although x-ray examination revealed a gross right basal lesion, probably encysted fluid. On culture tubercle bacilli were isolated from sputum.

Case 15.—A married woman was diagnosed clinically as suffering from severe bronchitis complicated by pyelitis. Ordinary physical examination revealed no sign of tuberculous disease of the lungs, and the bronchitis cleared completely following recovery from pyelitis. No x-ray examination was made. Culture of the sputum for tubercle bacilli was successful.

Case 16.—A married woman aged 32 was under observation for pulmonary tuberculosis, but no evidence was obtained other than the isolation of tubercle bacilli from a specimen of sputum. There appeared to be considerable doubt as to the diagnosis in this case, as x-ray examination carried out four years previously was negative but had not been repeated. The possibility of a right apical lesion was not entirely ruled out.

Case 17.—This male patient complained of symptoms indicative of an intrathoracic lesion on the left side. The sputum was blood-stained, but showed no tubercle bacilli in direct films, although a growth of this organism was present on

culture. The x-ray appearances suggested a neoplasm of the lung. The patient died in hospital, but permission for a post-mortem examination was not obtained.

Discussion

The results of this investigation over a period of two years have justified a cultivation test in the routine examination for *B. tuberculosis*. The adoption of cultivation lengthens the examination considerably, and on this account careful choice should be made of the material subjected to the treatment. This entails the co-operation of clinicians, particularly in the case of laboratories dealing with large numbers of specimens from hospital sources. Much of the time and energy now expended on multiple routine examinations, often demanded without real justification, could be profitably devoted to a more prolonged investigation of a smaller number of likely cases.

The results of sputum examination were especially significant. Animal inoculations of all such specimens is rarely possible or even practicable, and in the absence of this test cultivation affords a very satisfactory substitute. In this series cultivation yielded an increase of 32.4 per cent. of positives over the direct film result. There is the possibility that multiple film examinations may have been more successful than the use of a single film, but it is extremely doubtful if the results secured by cultural methods could have been attained. That direct film examination is not sufficient has been indicated by Jamieson (1936), who found cultivation successful in 100 sputa from 100 known cases of phthisis, whereas repeated examinations of direct films were positive in but ninety-two cases. It is an additional advantage, moreover, in doubtful clinical cases, that the pathogenicity of the organism isolated from sputum can be confirmed if necessary. The routine procedure now adopted in this laboratory as regards sputum examinations is to report positive film results as before. In the case of negative results there is added a note that culture is being attempted and will be reported later if positive.

But the efficiency of the test cannot be gauged merely by the increased number of positive results which it yields. There must be confirmation of the bacteriological findings by other methods. That confirmation has been amply obtained, as the majority of the cases giving negative direct film but positive cultural results, and for which clinical data were available, were unquestionably infected subjects. All stages of infection have been detected in this way. In a very small number of subjects there was not, in the clinician's opinion, much ground for supporting the bacteriological diagnosis, but the possibility of this being correct could in no case be excluded. When one remembers the prevalence of subclinical infection as indicated by post-mortem examinations, tuberculin testing, and other methods, it is not surprising that cases of this nature will be discovered by the cultural method. But the delicacy of the test calls for great care in the taking of specimens for examination, particularly in cases where the possibility of contamination with tuberculous secretions is present—as, for instance, the investigation of family contacts by the examination of sputa. Precautions must be made to ensure that only the subject's secretions and not those of a relative reach the specimen container. The method appears to be of value even in the early stages of infection, and may possibly be applied to detect an initial infection in childhood. Case 10 may prove to be an instance of this, as the child is now progressing satisfactorily. It may be stated here that there was no doubt that the growth was derived from the child's own secre-

tions and was of the human type. Shrewsbury has raised the point that the method may be applied to the detection of "carriers" of tubercle bacilli. While on account of the existing lack of information this possibility cannot be denied, it is more than likely that the so-called "carrier" is a person with a focus which defies clinical demonstration during life. Such cases can only be demonstrated by cultural or animal inoculation methods, so that the ultimate issue remains as before—namely, the possible detection of a hidden source of infection. One further point on which the method will furnish reliable information is the incidence of the two types of tubercle bacillus in human infections.

While not so striking on account of the smaller number dealt with, the usefulness of cultivation was also indicated in the examination of specimens other than sputa. At the present time, however, cultivation cannot entirely replace animal inoculation, particularly in specimens in which there is a possibility of the bovine type of organism existing in small numbers. Feldman (1934) after an investigation of similar nature came to the same conclusion. Animal inoculation cannot, however, be regarded as infallible, and in Table II appear four instances in which animal inoculation proved negative, whereas cultures from the same inoculum were positive. Examples of this type of result have been recorded by Woolsey (1931). The results of animal inoculation and of cultivation (Table II) were the same in ninety-nine specimens and different in nineteen. Of these nineteen, animal inoculation was most frequently positive (thirteen), while cultivation was successful in four specimens which were negative in animals and two specimens in which inoculated animals died before a report was permissible. The optimum results, then, can only be secured in the investigation of such material by the utilization of both methods of examination.

Conclusions

1. Cultivation is a practicable measure in the routine examination for *B. tuberculosis*.
2. In a series of 3,306 specimens of all types, mainly sputa, the percentage of positives was 12.9 by direct film and 17.9 by the combined film and cultural examinations.
3. While animal inoculation is in most cases more reliable than cultivation, it is not invariably so.

It is with pleasure that I acknowledge the assistance and willing co-operation of the laboratory technical staff. Without their help, and in particular that of Mr. R. K. Farmer, such a large number of specimens could not have been handled. I am indebted to Professor T. J. Mackie for his interest in the work and the granting of various facilities. To all practitioners who supplied clinical data my thanks are due, for their information added a very real interest to the investigation.

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