adult life in non-tropical countries. In common with the majority of cases already described our patient presents a persistent fatty diarrhoea, low serum calcium and phosphorus, actual or latent tetany, a flat blood-sugar curve after glucose ingestion, marked dilatation of the colon, and abdominal distension. She also shows achlorhydria and a moderate degree of glossitis, but no evidence of any marked anaemia.

In view of the recurrent tetany and the constantly low serum calcium, one would expect some evidence of skeletal decalcification, but the absence of this may perhaps be explained by the results of the calcium balance, which point to only a small loss of endogenous calcium. The nitrogen balance determination eliminates any possibility of the steatorrhoea being of pancreatic origin. Hess Thaysen has stressed the necessity of this investigation in the differential diagnosis of pancreatic and idiopathic steatorrhoea. Together with the urea concentration test the faecal nitrogen value indicates that, in contrast with the faulty absorption of fats and possibly of carbohydrates, nitrogenous products are efficiently absorbed by the patient. Under the existing circumstances one would look for some increase in the nitrogen metabolism, and it is probable that the loss of weight and the low serum protein value with its associated oedema are evidence of protein malnutrition, resulting not from any disturbance in digestion or absorption, but from a relative insufficiency of protein intake.

The interpretation of the results of the inquiry into the nature of the faecal fat is admittedly difficult, owing to lack of published data. The character of the excreted fat differs considerably from that of the ingested butter, indicating that total faecal fat measurements cannot be regarded as a quantitative index of the absorptive power of the intestine towards fats. The fact that the iodine value of the faecal fat is lower than that of the ingested butter is suggestive of its being derived from the food and not from endogenous fat, the iodine value of which is much higher. This would support the usually accepted theory that the steatorrhoea in such cases is due to deficiency in intestinal absorption rather than to the alternative possibility of derangement in the mechanism of excretion from blood into the intestine.

Treatment of our patient has been disappointing. In spite of adequate doses of calcium, vitamin D, hydrochloric acid by mouth, and a low fat diet relapses continue to occur, and it appears that the only measure which checks the diarrhoea effectively is complete rest in bed.

Summary

1. A case of idiopathic steatorrhoea originating in adult life is described.
2. A surprisingly good general condition of the patient and absence of bone decalcification in spite of persistently low serum calcium are noted.
3. Evidence is brought forward showing that absorption of fat and carbohydrate is inefficient, while that of protein is normal.
4. Persistent oedema associated with low serum protein is observed, and an explanation for this is suggested.

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THE CULTIVATION OF MYCO. TUBERCULOSIS FROM HUMAN SPUTA

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The routine laboratory examination of sputum from suspect cases of pulmonary tuberculosis consists of the microscopical examination of appropriately stained films of the sputum. This is the only method recognized by the tuberculosis authorities for which they accept the financial responsibility. Every laboratory worker knows that it is only of use in positive cases and that a negative microscopical report on a single sample of sputum is of no diagnostic value. It is interesting to compare the reliance placed on the microscopical method in tuberculosis to the present position in regard to the laboratory diagnosis of diphtheria, yet there is no valid reason why cultural examination of sputum and other morbid materials for *Myco. tuberculosis* should not be practised as a routine procedure and be recognized as equal in value to the cultivation of *C. diphtheriae* from throat swabs, etc. Given a reasonably simple and effective technique, the examination can be incorporated in the ordinary routine work of the laboratory; *Myco. tuberculosis* is not a difficult organism to cultivate, though it is a slow grower even under optimum conditions.

We have tested the following technique over the past two years and have found it suitable for the routine cultural examination of sputum. Before incorporating the method in our routine work we carried out a large number of control cultivations on morbid materials in which *Myco. tuberculosis* was found by microscopical examination. This method yielded 100 per cent. positive cultural isolations from tuberculous sputum, cerebro-spinal fluid, faeces, urine, and pus. Having thus established to our satisfaction the value of the method, we then proceeded to apply it to the examination of a series of sputa that were negative for *Myco. tuberculosis* on microscopical examination.

Technique

Any volume of sputum up to a maximum of about 5 c.cm. is thoroughly mixed with an equal volume of 4 per cent. caustic soda solution. The mixture is incubated at 37° C. for thirty minutes and is then centrifuged—in our case for one hour at 3,000 r.p.m. (The speed and time of centrifugation can probably be safely varied to suit individual convenience so long as the entire quantity of particulate matter is firmly packed at the bottom of the tube.) The supernatant fluid is then carefully decanted and the deposit rendered neutral to litmus with 4 per cent. hydrochloric acid. As a rule about five drops of acid are required to neutralize the deposit from an initial volume of 5 c.cm. of sputum. The deposit is then thoroughly mixed up with a sterile glass rod and is thickly sown on to the selected medium.

Three media have been tested in the course of our work—namely, Löwenstein's, Petroff's and Petragnani's—employing the method of preparation described by Jamieson (1936). All three media have given successful results, but we have found Löwenstein's to be the best for routine use. In our experience different strains of *Myco. tuberculosis* have varied in their preference for these media, but

all the strains we have isolated have grown readily on Löwenstein's medium. The way in which the medium is used appears, however, to be a determining factor in the cultivation of the bacillus in some cases at least. Medium slanted in test tubes is generally unsatisfactory, and has often given no growth when parallel cultures put up in screw-capped bottles, as recommended by McCartney (1934), have shown good growth. We therefore strongly advocate the routine use of these bottles as containers for the selected medium. The cultures are of course incubated at 37° C. Growth is usually visible in twenty-one days, but even when no colonies are visible at the expiration of this period there may be a good growth of *Myco. tuberculosis* as an extremely thin film on the surface of the medium. No cultures should therefore be discarded as negative until they have been examined microscopically, since transplants from these film growths grow vigorously and typically.

In our series of negative microscopical sputa examined culturally we have included only those which contained purulent matter; purely mucoid and salivary specimens were not considered. Up to the present we have examined 135 specimens, from which we have cultivated acid-fast bacilli—with the morphological and cultural characters of *Myco. tuberculosis*—in twenty-nine cases (21.4 per cent.). Most of these cultures have not yet been subjected to the biological test, but two of them have been inoculated into guinea-pigs because their extreme chromogenicity gave rise to some doubt about their identity. In both cases the inoculated animals showed the characteristic picture of experimental tuberculosis when they were killed, and typical acid-fast bacilli were found in film preparations from the affected lymphatic glands.

Results of the Investigation

From the above findings it is evident that in 21 per cent. of cases the ordinary routine laboratory test for pulmonary tuberculosis failed to give information that is of vital importance both to the individual and to the community. Tubercle bacilli can only be discovered with certainty by microscopical examination when they are present in large numbers in the sputum, but cultivation can detect them when they exist in very small numbers. In the early stages of pulmonary tuberculosis the bacillus will only be found in small numbers in the sputum. These are just the cases in which a positive laboratory diagnosis is of the greatest value, and which are most likely to be missed by a purely microscopical examination.

The positive cultural findings in most of our twenty-nine isolations were accepted by those who submitted the specimens to us as agreeing with or confirming their clinical findings. In three cases, however, we were informed that there was no clinical evidence of phthisis, and that the cultural findings could not be accepted. We were not informed why, in the complete absence of any suspicion of phthisis, the sputum should nevertheless be sent to the laboratory for examination for *Myco. tuberculosis*, but possibly the examination was requested as a placebo for the relatives. Assuming that in these three cases the clinical opinion is correct, and that no focus of tuberculosis exists in the thorax, the isolation of the bacillus from the sputum raises the very interesting possibility of the "carrier," either temporary or permanent. Carriers of *Myco. tuberculosis* certainly exist, and in a population with a high degree of acquired resistance they should theoretically be numerous, but their relation to the present incidence and distribution of tuberculosis is not known and is never likely to be known so long

as the microscopical test is accepted as the routine procedure by the tuberculosis authorities. The institution of a routine cultural examination of all purulent sputa that are microscopically negative would, we believe, throw light upon this important problem and lead to the recognition of those early cases in which there is some hope of permanent cure by efficient treatment.

The Fermentative Reactions

An additional feature of our study has been an examination of the sugar fermentation reactions of *Myco. tuberculosis*. Our investigations are at present not far advanced, but as they may be of some value to workers interested in systematic bacteriology, and as the pressure of routine work makes it extremely unlikely that we shall be able to complete them in the near future, we include them here. The lack of information about the fermentative characters of *Myco. tuberculosis* has been due to the difficulty experienced in finding a simple yet satisfactory basic sugar-free liquid medium that will support the growth of the bacillus. One of us (J. B.), after numerous trials, has evolved a medium which appears to satisfy the necessary requirements. This medium is prepared as follows. The white of one egg is thoroughly mixed with 200 c.cm. of tap-water. The mixture is then boiled for five minutes, filtered through a thin layer of cotton-wool, and cooled. One per cent. of sugar and 3 per cent. of litmus, or 1 per cent. of Andrade's indicator, are added, and the medium is put into small screw-capped bottles containing gas tubes and sterilized. In this medium most of the strains of *Myco. tuberculosis* that we have tested have grown slowly but quite vigorously at 37° C. The fermentative reactions have begun after some three weeks' incubation. We have kept our cultures for an arbitrary standard period of six months, comparing them with uninoculated controls, none of which has shown any appreciable change in reaction over that period. Up to the present we have tested thirty strains of *Myco. tuberculosis*, isolated from various human sources, upon the four sugars, dextrose, lactose, mannite, and saccharose. Four of these strains failed to grow in any of the sugar media. Of the remaining twenty-six all have produced acid without gas in lactose, twenty-five have produced acid without gas in dextrose, twenty-one have given the same reaction with saccharose, and three have attacked mannite.

We wish it to be clearly understood that our investigations are only in a preliminary stage; we have not had an opportunity of checking them properly, and we are therefore not certain that they are accurate. We hope, however, that some other worker with more time for research may be sufficiently interested to pursue this investigation further.

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Dr. C. Claoue (39, Rue Scheffer, Paris) has resumed the issue of the periodical *L'Activité Scientifique et Médicale* in typescript form. The May number contains a brief account of the German Congress of Surgery in Berlin from March 31 to April 3, a programme of the coming Reunion Médico-Chirurgicale de Morphologie in Paris on May 19, a summary of the contents of the fourth (December, 1936) bulletin of the last-named society, and an announcement of the arrangements being made to promote associations between the universities of France and Germany.