REPORT ON FURTHER OBSERVATIONS ON BACILLUS VIRIDANS.

[From the Pathological Laboratory of the University of Aberdeen and the
Institut Pasteur, Paris.]

By WM. ST. CLAIR SYMMERS, M.B. ABERD.,
Pathologist, General Hospital, Birmingham.

Pleomorphism.—At the date of the first communication¹ I had observed the microbe under various guises—bacterial, bacillar, filamentous—and since that time I have seen it assume almost every known form. There is a short rod form whose diameter is half its length; there is a bacillus whose length is $2\frac{1}{2}$ to 4 times its diameter; there are short threads whose length varies from 8 to 20 times the diameter. There are filaments, wavy as a rule, which stretch across the whole field of the microscope. These long solid threads often entangle themselves into beautiful spirulina forms, like ropes twisted of two strands. Filaments are seen which taper at both ends, running away to scarcely perceptible points; others are of equal diameter throughout. Vibrio forms, wavy and snake-like, have been seen. In various cultures a beautiful spiral of 8 to 10 turns has been observed. Comma forms and spirals of one turn were numerous, especially when cultivated in Koch's gelatine plus $\frac{1}{2}$ per cent. of boric acid. Chains of bacilli occur when a little gentian violet is added to the nutrient medium. In a twelve days' culture, to which boric acid had been added, beautiful cocci were developed. Lastly, $\frac{1}{2}$ per cent. sodium formate added to nutrient gelatine furnished an enormous number of spore-bearing filaments. These observations were checked by fractional cultivations. And no more convincing example of pleomorphism could be demanded.

Abeysance of Pigment Formation.—While the microbe is restrained by various antiseptics from secreting pigment, it still retains pigment-forming properties and needs only a suitable concurrence of circumstances to render these apparent. Although boric acid inhibits its pigment-producing faculty, the culture removed from its influence and transferred to bouillon at once displays its green colour as beautifully as before. If, however, the microbe is passed through the system of the rabbit, it loses permanently its power of producing pigment in bouillon. If now this "race" or altered form is inoculated on Koch's gelatine it reassumes its chromogenic power. These two peculiarities are the same as those which M. Gessard has so beautifully shown as appertaining to *B. pyocyaneus*.

Relation to Aniline Dyes and Coloured Media.—The microbe is immediately decolorised by the alcohol in Gram's method. Gentian violet stains it deeply, methylene blue very faintly. The affinity for aniline dyes, however, varies according to the medium upon which the microbe has been nourished. Thus the organism grown on cocoa-nut stains very faintly by Kühne's methylene blue, whereas the same solution stains most intensely those microbes which were grown on carrot. The same holds good of Kühne's solution of gentian violet in regard to the growth on beetroot and parsnip. Such instances could be multiplied, and seem to indicate that the microbial protoplasm varies according to the nutritive media employed. The bacillus grows well on media coloured by aniline dyes, and never under these conditions takes on the dye. The tinted media become decolorised, or altered in colour by the microbe. Thus aniline green completely loses its colour. The action of the organism on methyl blue is very remarkable. Tubes of Koch's gelatine deeply tinted by this dye completely lose their colour except at the surface, where there remains a mere film of dusky green. On agitation, the whole medium becomes of this green hue, but when left at rest the fluid once more becomes colourless with the exception of the surface layer. This phenomenon may be recalled as often as desired, and evidently depends on the action of oxygen on the liquefied mass.

Effect on Animals.—Rabbits, guinea-pigs, mice, pigeons, etc., fed upon enormous masses of the microbe remained unaffected. Subcutaneous injections in large quantity into

white mice, white rats, guinea-pigs, and pigeons were harmless. Guinea-pigs after injections into the peritoneal cavity invariably suffered from a severe malady from three to five days. They became much depressed, remained in one place, ate nothing, were evidently in pain if touched on the abdomen, and their temperature was somewhat elevated. It is noteworthy that, after recovery, no amount of intraperitoneal injection produced even the slightest *malaise*, even when daily repeated in large quantity. By far the most interesting result was obtained with rabbits. Subcutaneous injections on the trunk were harmless; intraperitoneal injections were followed by slight and transitory *malaise*, while intravenous injections (40 m distilled water made milky by the microbe) occasionally caused death in from thirty-six to forty-eight hours, but left no organic changes recognisable after death.

The pathological effect *par excellence* was, when the microbe was injected subcutaneously over the ears, the production of baldness. This was easily elicited in all the rabbits experimented upon—twenty-five. In about ten days, as a rule, the hairs commence to fall out, and gradually the spot of baldness widens until the area comes to present exactly the appearance of a patch of alopecia areata in man. After several weeks the hairs reappear, and ultimately the baldness, as is the case in human alopecia, completely vanishes. I have produced this alopecia two and three times in the same ear of the same rabbit, so that one attack does not confer immunity against a second. This baldness is produced by small quantities of the microbe by 5 m of a milky suspension of the organism in distilled water. If 20 m or more are injected there arises a severe inflammation, the whole ear is much swollen, and hangs heavily against the side of the animal's head. In a few days the tissues slough away in such a manner as to leave an oval perforation from 1 to 2 inches long. In time the inflammation subsides, the hairs grow to the margin of the perforation, which remains permanently open, and looks as if it had been clearly punched out by some sharp instrument. These effects could not be produced with cultures of the bacillus which had been sterilised by heat or by passage through Chamberland's filter.

I desire, in conclusion, to return my warmest thanks to Professor D. J. Hamilton, and to express my admiration for the unrivalled facilities of the Institut Pasteur.

PRELIMINARY REPORT ON THE GLYCOLYTIC FERMENT OF THE PANCREAS.

By E. MANSEL SYMPSON, M.A., M.D. CANTAB., M.R.C.S.,
Surgeon to the Lincoln County Hospital.

UPON consideration of the remarkable experiments of Lépine, Minkowski, and others, on the presence of a glycolytic ferment in the blood, and the apparent dependence of this ferment in great measure on the presence or absence of the pancreas, as well as the well-known pathological connection between disease of that organ and diabetes mellitus, it seemed reasonable to hope that a substance of the nature of a glycolytic ferment could be discovered in the pancreas itself. Another function of the pancreas appeared to be necessary, the production of a ferment which would bring about the glycolytic changes in the blood. Obviously, it would not be poured out, in all probability, into the common duct, as the ordinary amylolytic ferment would neutralise its action, but it would pass away from the pancreas by the portal vein. In other words, as Dr. Osler has neatly put it:—

The pancreas on this view has, like the liver, a double secretion, an external, which is poured into the intestines, and an internal which passes into the blood. This latter is supposed to be of the nature of a ferment, in the presence of which alone the normal assimilative processes can take place with the glycogen. Disease of the pancreas causes diabetes by preventing the formation of the glycolytic ferment.¹ This is rendered still more probable by Minkowski's² later experiments in the prevention of diabetes after extirpation of the pancreas by transplanting a piece of the organ to another part of the abdominal cavity, thus showing clearly that as long as even a small portion of the gland retained its vascular

¹ BRITISH MEDICAL JOURNAL, December 12th, 1891.

² BRITISH MEDICAL JOURNAL EPILOGUE, December 10th, 1892.

connections the ferment continued to be sent into the blood, and so diabetes was prevented. One or two objections which have been raised to this line of experiment may be usefully considered before detailing the experiments which are the subject of the present paper. One is that the glycolytic changes described by M. Lépine are *post mortem*, and probably do not occur during life at all. The evidence about to be given will, I trust, show that there is as much reason to believe in this glycolytic ferment of the pancreas as in the peptonising or amylolytic ferments of the pancreatic juice, save that it has not been obtained from a fistula in the pancreatic duct.

Secondly, it is urged that the pancreatic origin of diabetes does not explain all the cases of diabetes. He would be a very unscientific observer who should affirm the contrary, like a eulogiser of a patent medicine. It is said that when in a case of diabetes you have cut off all carbo-hydrates from your patient's diet, still there goes on this excessive glycosuria, which is accompanied by an excessive excretion of urea. Therefore they say, if the glycosuria simply depends on a failure of the system to destroy sugar, the amount got rid of should be proportional to the amount received as food, and no account is taken of the output of urea. This objection suggests some interesting features of diabetes. First of all, there are probably many cases of diabetes wherein the pancreas is not diseased at all. These are chiefly cases of glycosuria occurring in elderly patients who may be gouty, and not infrequently distinctly corpulent. In these very probably the source of much of the sugar is to be found in the muscles, and so not so much exposed to the action of the pancreatic ferment. In other cases of diabetes, generally among the young, there have been found certain symptoms, more or less marked, of affection of the pancreatic gland. Emaciation with great voracity, fatty stools, the passage of undigested meat, and probably pallor of the stools, are all symptoms of disease of the pancreas, and have all been noted in a relatively large number of cases of diabetes. Now it may fairly be suggested that in a typical case of diabetes with these symptoms, the action of the pancreatic juice being diminished or abolished, "the sugar may arise from proteids which have been split up into a nitrogenous (urea) and a non-nitrogenous moiety" the sugar. But if this were all there need surely be little or no glycosuria; the extra supply of sugar would be stored up as usual in the liver. But, granting the existence of a glycolytic ferment also in the pancreas, this naturally is interfered with also by the disease of the gland, and permits the sugar to circulate in the blood, and, of course, to be excreted by the kidneys.

EXPERIMENTS.

Only a brief account will be necessary on the present occasion. The material was always a freshly killed sheep's pancreas. First of all some experiments were made to see if there was any glycogen in the pancreas; not a trace could be found. Next experiments were made, and are going on, to obtain a glycolytic ferment from the pancreas. The same results were gained invariably. For instance on November 26th two test tubes with a solution of glucose (3gr. to 3ss) in each, and in one an addition of 3j of an aqueous solution of pancreas were kept in an incubator at a temperature of 100° F. for ten hours. On being examined with Pavy's modified Fehling's solution, the one with the pancreatic solution showed much less sugar than the other.

On November 28th, a test tube with the same amount of glucose solution and 3 drops of a glycerine extract gave much less evidence of sugar than a similar tube exposed to the same temperature of 100° F. for twelve hours. These tests were also vividly confirmed by Moore's test, and later on by Oliver's. In an experiment for twenty-four hours, the difference between one solution and the other (which had been exactly the same) was equal to 50 per cent. Also by Zeiss's saccharimeter a difference of one degree and a half was found under similar circumstances. The solution was also tested to see whether any carbonic acid gas was given off during the process; no trace of the acid could be found. Also, to show that the glycolysis was due to a ferment, the solution of pancreas was thoroughly boiled before it was added to the glucose solution, and this completely checked all glycolytic action.

The glycerine extract of pancreas seemed more active in producing glycolysis than an aqueous extract, and is, of course, much more easily kept. Glycerine itself, in exactly the same doses as were given of the glycerine extract of pancreas, had no effect whatever.

Finally, then, there seems to be no doubt that a substance can be extracted from fresh sheep's pancreas, which is of the nature of a ferment, and which has a powerfully glycolytic action on solutions of glucose. An objection (which I hope very shortly to undermine) may indeed be raised that the glucose used in these experiments was not absolutely pure. Still this does not outweigh in my opinion the facts that again and again the extract of pancreas (whether glycerine or aqueous) has destroyed a quantity of sugar in one tube, when an exactly similar tube, exposed to the same conditions of temperature and measurements (only without the pancreatic extract), has kept of exactly the same strength as it was at the beginning of the experiment.

If, then, these various symptoms of disease of the pancreas in diabetics be correlated with a glycolytic ferment in the pancreas, the important bearing on treatment is, as I wrote in the *Practitioner* (August, 1891), to give these patients, as has been done with some success, liquor pancreaticus and the sweetbreads of calves and other animals.

My thanks are due to the practical encouragement afforded me by the Scientific Grants Committee of the British Medical Association.

CASE OF MYXCEDEMA TREATED BY THYROID FEEDING.

By CONSTANTINE HOLMAN, M.D.,
Vice-President of the British Medical Association.

Mrs. B., aged 44, came under my care on October 3rd, 1892. She had been suffering from symptoms of myxœdema, as recognised by Dr. Ord seven years since.

CONDITION AT COMMENCEMENT OF TREATMENT.

Face, hands, feet, and body swollen; features expressionless. Forehead deeply lined; hair frizzy. Skin generally cold, dry, and unsperspiring, and feeling hard and even horny in some parts, such as the palms of the hands, soles of the feet, and forehead. On account of the swelling she was unable to button her boots or to wear any but very large gloves, or to wear her usual dress. The mucous membrane of the mouth was red and swollen, overlapped the plate of the false teeth, and bled on the slightest pressure. Hæmorrhage difficult to restrain followed extraction of a tooth. Pain over region of right kidney. Urine—specific gravity 1024—contained blood and pus corpuscles in considerable quantities; one-sixth albumen. Free menstrual loss every second month.* Articulation slow, imperfect; thinks very slowly; writes with difficulty; spelling defective; memory very bad. Breathless on slight exertion. Restless; unable to apply herself to read or to work. Pulse 64, temperature 96.6°. In consultation with Dr. Ord, it was decided to treat the patient with sheep's thyroids, one whole gland—both lobes—to be taken every other day.

PROGRESS OF CASE.

Treatment was commenced on October 12th. The remedy took immediate effect—the pulse rose from 64 to 96 on October 14th, on the 18th to 106, and on the 21st to 130. The temperature, from 96.6°, rose to 97° on the 14th, and to 97.4° on the 18th, and 98.8° on the 21st.

Complaint was made of tumultuous action of the heart at night; she slept badly, and was reported to be talkative in her sleep. More urine passed; it contained much less blood, less pus, less albumen, but a large amount of mucus.

On October 21st all symptoms better, skin perspiring, face less swollen; more expression in features; hands and feet less swollen, less chilly; more power of application in reading and writing; urine contains no blood, very few pus corpuscles, less albumen; large quantity of mucus; pulse 130, temperature 99°. As the patient complained much of the action of the heart at night, the thyroid was omitted for a few days, and then a much smaller amount was given—half a lobe every other day. The pulse rate soon diminished in

* Foster's Physiology, p. 734, 1888.