in children and young persons which have seemed to me to point very strongly to the correctness of the inferences of Potain and Samways. It is the rule to find in the *post-mortem* examination of such cases that the left ventricle is of small capacity contrasting with the large dilated right ventricle of the same subject, and with the normal left ventricle of a child of the same age as the subject observed. There cannot be any doubt that the morbid condition of mitral stenosis is of slow pathogenesis. These morbid processes must have commenced at the periphery of the valve curtains, or at the auriculo-ventricular orifice, and it seems to me that unless some cause prevented regurgitation, regurgitation must have occurred. If such regurgitation had occurred, why is it that its usual consequences are not in evidence? Surely the enhanced blood pressure within the ventricle would have caused left ventricular dilatation. But such dilatation is conspicuous by its absence. It is in the highest degree probable therefore that regurgitation was prevented, and, according to the accumulated testimony, prevented by the action of the auricle.

It is probable that the normal endowment of the auricular muscle is sufficient to prevent the regurgitation in the earlier stages of development of mitral stenosis, but in course of time the muscle becomes hypertrophied. Later on the dilatation of the auricle preponderates, and the muscular fibres become attenuated to almost extinction. Atrophy of the auricular muscle may, however, occur in the earlier stages from various causes of faulty nutrition. My own experience coincides with that of Dr. Samways in establishing as a general rule the doctrine that "the characteristic alteration in the left auricle in mitral stenosis is not dilatation but hypertrophy—dilatation is probably introduced only with the auricular breakdown."

The prognosis in the condition of disease which we are considering is grave. I have found the average age at death of 61 patients to be 32.7 years, but it is to be remembered that my cases included an unusual number of children, for in the etiology of the disease in children I was much interested, and probably more than my fair proportion of cases were sent under my notice. The late Dr. Hayden's cases, 42 in number, gave 37.8 years. Sir William Broadbent, 53 cases, gives as the average age at death 33 years for males and 37 to 38 for females.

The most recent inquiry, that by Dr. Samways¹⁷ as to the *post-mortem* records of Guy's Hospital during ten years shows that the average age at death, males and females (identically) is 38½ years; the less pronounced forms of constriction of the orifice 43.6 years; the more extreme stenosis 33.6 years.

The concurrence of testimony, therefore, is to show that the conditions of the disease are so grave that life is rarely prolonged beyond 40 years. In the form of disease which is associated with chronic fibrosis and granular kidney, however, the age at death is much more advanced; but these commence insidiously for the most part at a comparatively advanced age. I have observed such a case at the age of 70.

NOTES AND REFERENCES.

¹ Duroziez, P., *Traité Clinique des Maladies du Cœur*. Paris: Steinhell. Pp. 258 et seq. ² Hayden, *Diseases of Heart and Aorta*, p. 942. ³ Potain, C., *Clinique Médicale de la Charité*. Paris: Masson. 1894. Pp. 178 et seq.; pp. 507 et seq.; Teissier, Pierre, *loc. cit.*, pp. 913 et seq. ⁴ Blind, F. A., *Le Rétrécissement Mitral des Artério-Scléreux, Thèse de Paris*. 1894. ⁵ Samways, D. W., *Le Rôle de l'Oreille Gauche notamment dans le Rétrécissement Mitral*. *Thèse de Paris*, 1896. ⁶ Gérard, L'Oreille Gauche dans le Rétrécissement Mitral, *Thèse de Paris*, 1894. ⁷ *Ibid.*, p. 65. ⁸ *Ibid.*, p. 17. ⁹ Sansom, *Transactions of the Medical Society of London*, 1881, pp. 197-207. ¹⁰ *Ibid.*, *Diseases of the Heart and Aorta*, p. 378. ¹¹ Potain, *Du Cœur de la Pointe du Cœur*. ¹² Woods, Robert H., M.B., F.R.C.S., Letter in BRITISH MEDICAL JOURNAL, March 6th, 1897, p. 627. ¹³ Marc Sée, Sur le Mode de Fonctionnement des Valvules Auriculo-Ventriculaires du Cœur, *Arch. de Phys.*, 2ème série, tome I, p. 381. ¹⁴ Roy and Adami have been able to detect this interval. Soltan Fenwick and Overend found that the papillary muscles contracted exactly at the same moment as those of the ventricular wall. *Lancet*, October 26th, 1889. ¹⁵ Gérard, *loc. cit.*, p. 29. ¹⁶ Landois and Stirling, *A Textbook of Human Physiology*. London: Chas. Griffin and Co. 1885. Vol. I, p. 69. ¹⁷ Samways, BRITISH MEDICAL JOURNAL, 1897, I, p. 197, and 1898, I, p. 364.

THE lepers of the Orange Free State are in part placed on a farm in the neighbourhood of Bloemfontein, in part maintained on Robben Island at the expense of the State. A proposal is now made that the lepers should be removed from the island and placed upon another farm in the Free State.

THE CROONIAN LECTURES ON THE CHEMICAL PRODUCTS OF PATHOGENIC BACTERIA CONSIDERED WITH SPECIAL REFERENCE TO ENTERIC FEVER.

Delivered before the Royal College of Physicians of London;

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LECTURE II.

WITH these preliminary observations we now pass to the consideration of the investigation more recently made on the chemical products of the typhoid bacillus, of the bacillus coli communis, and of a form allied to these, namely, the bacillus enteritidis of Gärtner.

These three micro-organisms are in culture closely allied to each other. They all grow in broth, forming a cloudiness and a sediment, not commonly a scum on the surface, although this is present sometimes in cultures of the bacillus coli. The growth on agar is very similar in all three forms, being more fluorescent with the bacillus coli than with the other two forms. On gelatine they grow without producing any liquefaction of the medium. Their differences are as follows: In glucose gelatine and glucose agar the bacillus coli and Gärtner's bacillus form numerous bubbles of gas, while the typhoid bacillus, although it grows copiously, produces no bubbles. The bubbles are due to the formation of gas from the sugar present. On potato the bacillus coli gives a fleshy yellowish-brown growth; the Gärtner bacillus a slightly coloured, and the typhoid bacillus gives a filmy—almost transparent—growth. Milk is coagulated by the bacillus coli, but is not coagulated by the typhoid bacillus or by Gärtner's bacillus. Indol is formed in a broth culture by the bacillus coli, not at all by the typhoid bacillus, and only a slight indol reaction can be obtained, and that not constantly, in cultures of the Gärtner bacillus. Some varieties of the bacillus coli do not appear to form indol.

These cultural characteristics are slight, but are sufficient for the purposes of diagnosis, inasmuch as the bacillus coli can always be distinguished from the other two by the gas it forms in glucose gelatine, by its coagulation of milk and by the indol it produces, whereas Gärtner's bacillus is distinguished from the typhoid bacillus by the fact that it forms gas in glucose gelatine. In any of the media the growth of the bacillus coli and of Gärtner's bacillus is much more copious than that of the typhoid bacillus. The bacillus coli is further distinguished from the typhoid bacillus by not giving the "serum reaction" with the blood of a typhoid patient or with typhoid "antitoxic serum." Dr. Durham states that Gärtner's bacillus sometimes gives a sedimenting reaction with typhoid blood or serum. All these organisms are flagellated, the typhoid bacillus containing the most numerous flagella, the bacillus coli the smallest number, and Gärtner's bacillus being intermediate. The close similarity in vegetative properties of these three micro-organisms rendered it advisable that they should be investigated together. They probably play very different rôles in the production of disease. The typhoid bacillus is the cause of enteric fever; the bacillus coli plays a great part in the production of peritoneal lesions proceeding from the intestine and inflammatory conditions connected with the pelvic and other abdominal organs; and the Gärtner's bacillus was originally found in the body of a man who had been poisoned by beef which also contained the bacillus. Dr. Durham thinks that it may be the cause of some cases of food poisoning. Both the typhoid bacillus and Gärtner's bacillus may therefore be considered as micro-organisms, chiefly infecting the intestinal tract, and the bacillus coli—though it is not known to produce any gross lesion in the intestinal tract—is a natural habitant in that region and may aid in intestinal infections.

THE TYPHOID BACILLUS.

The typhoid bacillus is found in enteric fever, in the intes-

tinal lesions, in the mesentery glands, and in the spleen. It is present, in some cases, in the motions of typhoid fever, and also in the urine, and has been occasionally found in the liver and blood. It is also present in some of the abscesses of remote parts which may be observed in the course of typhoid fever or during its convalescence. It is most readily separated from the spleen, and in the following manner: After the spleen has been removed wholly from the body, it is washed on the surface with boiling distilled water, followed by absolute alcohol. It is then incised with a sterilised knife. By means of a sterile platinum rod inserted into the substance of the spleen, inoculation of broth tubes or the surface of agar plates is made, and within 24 hours there is a copious growth of the typhoid bacillus. Another method is to remove a small portion of the splenic pulp by means of sterile instruments, and place it in 200 c.cm. of sterile distilled water in a flask which is incubated for 24 hours. The liquid is then brushed over the surface of agar plates, and in 24 hours a copious growth of the typhoid bacillus is obtained.

I have examined four spleens in this manner: one from the Middlesex Hospital, kindly sent me by Dr. Fardon; one from St. Thomas's Hospital, sent by Dr. Hawkins; one from Homerton Hospital, sent by Dr. Goodall; and one from University College Hospital, from a case under the care of Dr. Ringer. With the exception of one case all gave a pure and abundant culture of the typhoid bacillus. The exception showed a great preponderance of colonies of the typhoid bacillus on the agar plate, but one or two colonies appeared rather different to the others, being more opaque and fluorescent. These were separated, and proved to be the bacillus coli. All the remaining colonies, as well as those from the other three cases, gave all the typical reactions of the typhoid bacillus, including the serum reaction (Widal's). The presence of this pathogenic organism in pure culture in the spleen and its invariable presence is one of the arguments in favour of the typhoid bacillus being the cause of enteric fever.

INCREASE OF VIRULENCE OF THE BACILLUS.

In the investigation of the products of a pathogenic bacterium it is not of much value working with non-virulent cultures. None of the ordinary cultures of the typhoid bacillus obtainable in the laboratories will kill an animal. I obtained cultures from various laboratories in England, and found them all incapable of causing death. Although, as will appear, the action of the chemical products of these non-virulent forms are of some significance, yet much progress was not made until a virulent culture was obtained. Chantemesse and Widal¹ rendered the typhoid bacillus virulent by injecting 4 c.cm. of a broth culture under the skin of the guinea-pig, and at the same time injecting into the peritoneal cavity 8 to 10 c.cm. of the sterilised chemical products of the streptococcus. The animal died, and the typhoid bacillus was found in the blood, the organs, and in the peritoneal cavity. A culture was made from this in broth and used for a second experiment of a like character, employing rather less of the streptococcus products. When proceeding in this way it was found that less and less of the streptococcus products could be used, the animal still dying, until finally it was found that the culture of the typhoid bacillus obtained could, in subcutaneous doses of $\frac{1}{2}$ c.cm., kill the animal without the aid of the streptococcus products. Similar results have been obtained by using the products of the bacillus coli or of the bacillus prodigiosus instead of the streptococcus products. I attempted this method, using the bacillus coli products, but found that, although the virulence of the bacillus was undoubtedly increased, yet I could not obtain it in a virulent enough condition to cause death of itself. I obtained, however, an extremely virulent typhoid bacillus by utilising cultures made from the spleen of one of the cases examined.

The virulence of the typhoid bacillus as obtained direct from the spleen after death or during the course of the disease, varies somewhat. In one of the cases a small amount of splenic pulp was ground up with salt solution, and 2 c.cm. of this mixture were injected into the peritoneal cavity of a guinea-pig. Death occurred in the course of eight days, and a pure growth of the bacillus was obtained from the peritoneum, but not from the blood of the heart or the spleen.

From another case a small quantity of a similar mixture was injected into two guinea-pigs, and both died in under twenty-four hours, and a pure growth of the bacillus was obtained from the peritoneal cavity and from the blood of the heart. The first of these cases was used for obtaining a virulent bacillus; 4 c.cm. of a four-days old culture of the splenic pulp in broth were injected into the peritoneal cavity of a guinea-pig; death occurred in under twenty-four hours. There was slight effusion into the peritoneal cavity, from which a pure culture of the bacillus was obtained; $\frac{1}{2}$ c.cm. of this effusion was injected into the peritoneal cavity of another guinea-pig. Death occurred in under twenty-four hours, and in the subsequent procedure the effusion from the peritoneal cavity of one guinea-pig was injected into the peritoneum of another, death occurring in the twenty-four hours. At the eleventh successive inoculation, $\frac{1}{8}$ c.cm. of the peritoneal effusion killed the guinea-pig in two days. At the thirteenth inoculation $\frac{1}{4}$ c.cm. diluted in salt solution killed in less than twenty-four hours, and at the sixteenth inoculation half a platinum loopful of a twenty-four-hour-old agar culture injected into the peritoneal cavity killed a guinea-pig in under twenty-four hours.

During the course of these inoculations it was observed that there was almost constantly an exudation of turbid fluid of greater or less amount into the peritoneal cavity. There was occasionally a little lymph on the surface of the intestines, and sometimes there were ecchymoses in the spleen. The peritoneal exudation was highly albuminous, coagulating solid on heating. On microscopical examination it showed large numbers of short rods, more or less actively motile. In the first inoculations some leucocytes were present in the exudation, showing phagocytosis. Later on, however, as the bacillus became more virulent, the cells and phagocytosis were not observed, and the exudation consisted simply of a highly albuminous fluid containing enormous quantities of typhoid bacilli, sometimes in clumps, usually singly. As the virulence of the bacillus increased so did the time of death diminish, and at the twenty-eighth inoculation 2 c.cm. of the peritoneal exudation were injected into the peritoneal cavity of a rabbit, causing death in five hours and a half, a result which cannot be attributed solely to bacterial action, but must be ascribed to the chemical poisons present in the peritoneal fluid. This was very evident in the experiment on a rabbit weighing 590 g., which received 0.25 c.cm. of the peritoneal fluid into left marginal vein. Symptoms of collapse soon supervened, and death occurred in two hours and a half. The peritoneal exudation produced by the introduction of virulent typhoid bacilli is therefore highly poisonous, and these properties were investigated.

In all these cases the typhoid bacillus was obtained in pure culture in the peritoneal cavity, whether there was much or little effusion, and it retained throughout its typical cultural characteristics. There was no case in which there was a mixed infection with any other micro-organism, so that the effects were those of the bacillus itself. An examination was made of the distribution of the bacillus in the various organs of the body. In the earlier inoculations during the increase of virulence it was found that although the animal died the bacillus was limited to the peritoneal cavity, and was not present in the spleen or the heart's blood, showing that death was produced by absorption of the poisons formed by the bacillus in the peritoneal cavity, which acted as a sort of living test tube. In the later experiments, however, the bacillus did not remain in the peritoneal cavity, although its distribution throughout the body was not constant. Thus, in one experiment (peritoneal injection), at death it was found that the heart's blood, spleen, and liver all gave a pure culture of the typhoid bacillus, as well as the fluid in the peritoneal cavity. This was confirmed by the next experiment, but in the one following a pure growth was obtained from the peritoneal effusion and from the spleen, and not from the liver or the heart's blood. There is a tendency, therefore, more with the weak virus than with the strong, for the typhoid bacillus to limit itself in its growth to the seat of inoculation, although a general distribution of the bacillus throughout the body usually occurs with the virulent form. This limitation of the bacillus to the site of injection is well marked when it is subcutaneously in-

¹ Ann. de l'Institut Pasteur, vol. vi, p. 7:5, 1897.

jected. Thus in a guinea-pig which received subcutaneously a quarter of a cubic centimetre of virulent peritoneal fluid there was a swelling at the site of inoculation, which ended in a slowly forming abscess pointing in 11 days; a pure culture of the bacillus was obtained from it. The animal was killed on the twelfth day and a pure culture of the bacillus was obtained from the abscess, but the heart's blood, the spleen, the liver and peritoneum were sterile; the heart muscle was not degenerated. Chantemesse and Widal and others have, however, found that infection of the body can occur after subcutaneous injection of the bacillus, and this occurs readily if the process of infection be aided in the manner previously described.

It is interesting to note in this series of experiments the absence of leucocytes in the peritoneal exudation as the bacillus became more virulent; and the formation of an abscess and the limitation of the growth of the bacillus when the virulent virus was injected under the skin.

TOXIC ACTION OF THE PERITONEAL FLUID.

It has been stated that the cause of death when the virulent peritoneal fluid is injected into a rabbit or a guinea-pig was due more to the action of a chemical poison than to the growth of the bacillus, since this occurred so rapidly; in one case in five and a-half hours, and following intravenous injection in two and a-half hours.

The effects of this chemical poison were examined by filtering the peritoneal fluid through porcelain, in a small apparatus improvised for the purpose. In the first experiment $\frac{1}{2}$ c.cm. of this filtered sterile peritoneal fluid was injected into the marginal vein of a young rabbit. A moderate degree of fever followed the injection for several hours. Subsequently the temperature fell to the normal, and no symptoms were observed in the animal, which died at the end of thirty days. The heart's blood was sterile, and the heart muscle showed well-marked degeneration of the fibres—there being loss of striation with longitudinal fibrillation, and the presence of numerous small granules staining black with osmic acid. Other experiments were done in a similar manner, using 1 c.cm., 1.5 c.cm., and 2 c.cm. of the filtered peritoneal fluid. In all cases some degree of fever followed the injection, but no effect was subsequently observed, and death did not occur. The filtered peritoneal fluid was therefore not nearly so poisonous as the unfiltered; evidently the porcelain filtrate had kept back the greater part of the chemical poison, whether this was present in the albuminous fluid or in the bodies of the bacilli.

EXAMINATION OF THE POISON IN THE FRESH STATE IN THE SPLEEN.

Before continuing this part of the subject, we must return to the consideration of the poisons present in the spleen of typhoid patients. In all the cases examined, as has been stated, the typhoid bacillus was present in large quantities, and in only one was there an admixture with another bacterium, the bacillus coli.

The splenic pulp was rubbed through sterile wire gauze and treated with about 500 c.cm. of sterile normal salt solution. This was allowed to stand a short time, and an attempt was made to filter it through porcelain. This was a matter of great difficulty, only a small amount of the liquid passing through. It was thin and deeply blood-stained. It was injected into the marginal vein of a rabbit on two consecutive days—4 c.cm. on the first day and 3.5 c.cm. on the second day. No appreciable effect on either the temperature or the body weight followed the injection on either day or on the subsequent two days; but on the fifth day of the experiment the temperature was found to have fallen to 97.6° , and the animal was collapsed and had profuse and mucoid diarrhoea. The temperature remained subnormal the whole of that day, and at 10 o'clock on the sixth day it was 95.4° , and the animal was lying motionless on its side, but breathing regularly. It was killed with chloroform. At the *post-mortem* examination there was subcutaneous hæmorrhage over the lower third of the abdomen, extending down over the upper third of the front of the thighs. In the abdomen there was a soft blood clot, matting together the omentum and the adjacent coils of the intestine. There was no peritonitis. The thoracic organs were normal,

the intestines were empty, except the upper part of the colon and cæcum, which contained soft fæces. There was no enlargement or redness of the Peyer's patches or of any other part of the mucous membrane. From the peritoneal cavity was obtained a growth of the bacillus coli, but the spleen contained no micro-organisms.

What is interesting in this experiment is that, following the injection of the poison, there was a period of incubation, during which no effect on the body temperature or the weight was to be observed, and that a fall of temperature, associated with great bodily prostration and mucoid diarrhoea, supervened somewhat rapidly. This fall of temperature and the collapse and diarrhoea observed are, as will be seen, to be ascribed to the action of the chemical poison of the typhoid bacillus.

A more extensive chemical examination of the spleen was made by means of the precipitation of the extract with alcohol in order to obtain any albumoses that might be present, as well as to separate the alcoholic extract. The method has already been described. The amount of albumoses obtained from each of three spleens was 0.652 g., 0.37 g., and 0.369 g. When dried they formed a fine whitish yellow and very light powder, soluble in water, and giving a brownish solution, which gave a distinct biuret reaction. The alcoholic extract was yellowish in colour, and was separated from the fatty bodies by precipitation with a large excess of ether. It contained a very large quantity of leucin and tyrosin, which separated out in the form of crystals on evaporation. The final alcoholic extract was a yellowish, sticky, resinous-like substance when dried over sulphuric acid.

The injection of the alcoholic extract into the vein of a rabbit did not seem to produce any effect on the temperature, and no appreciable effect on the body weight. In one case 0.112 g. was injected, and the animal appeared as well after the injection as before; it did not die. There was no diarrhoea, nor were there any symptoms to be noted. The injection of the albumoses also had but slight effect on the animal. In one case 0.2785 g. was injected in three doses into a rabbit weighing 790 g.; a dose of 0.352 g. per kilo. of body weight. After the first injection of about 0.4 g. there was no effect on the temperature. After the second injection of about 0.12 g. there was a rise of temperature, the highest being 105° during the day. After the third injection on the following day of the same amount there was only a slight rise of temperature, but on the succeeding days it was again normal. No symptoms were produced in the animal by these injections except a slight rise of temperature. This effect, therefore, is a very different one from that observed after the injection of the saline extract in a fresh spleen. It may have been, of course, that the poison was destroyed by the alcohol during the course of preparation. This, however, is unlikely, inasmuch as the manipulation was very rapid, the products being separated within three days, and the action of the alcohol on the albumoses being less than 18 hours.

THE ACTION OF CERTAIN DRUGS AND MINERAL WATERS ON THE SECRETION AND COMPOSITION OF HUMAN BILE: AN EXPERIMENTAL INVESTIGATION.

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[ABSTRACT.]

THE investigation of cholagogues is one of the most difficult in the whole domain of pharmacology. The importance of the subject is shown by the number of laborious researches undertaken to determine the action of drugs which are supposed to influence the secretion of bile, and its complexity by the contradictory results obtained. As nearly all recent experimental evidence casts doubt on the existence of so-called cholagogues, it occurred to me that the time would be well spent if I could contribute, even in a minor degree, towards a solution of this very difficult problem. While arrangements were being completed for carrying out a series of investigations on dogs by means of Schiff's amphibolic