Control there were over 12,000 registered mental defectives, including criminal and non-criminal.

Certainly my suggestion is more humane than that of Plato, who, whilst not being opposed to marriages, wished that the offspring of the least worthy should not be reared; or of Aristotle, who was in favour of allowing children in excess of those required to die from exposure, and that all deformed children should not be permitted to live.

In conclusion, I may say first, that, as the mortality amongst illegitimate children is so great, it would be an advantage if an effort were made to save some of their lives, and if philanthropic arrangements could be inaugurated for bringing them up in healthy surroundings, so that they might eventually become of service to the State.

Secondly, that looking to the amount of sterility, and the enormous loss of life caused respectively by gonorrhoea and syphilis, it is urgently desirable that notification of these diseases should be rendered compulsory.

Thirdly, that, as I have pointed out, we have a distinct fall in the birth rate; that by the widespread adoption of contraceptives, which from a physiological point of view cannot be approved, but which we are certain will be ever increasingly used, we must not expect an average of more than two births from each marriage—possibly only one; that, as we know from a most conservative estimate there must be at least four children per marriage amongst families who can produce children, allowing for infantile mortality, those who never marry, and those who are unfit to produce children, we cannot anticipate a real increase in the population.²³ It follows, therefore, that our country is faced in the future with the problem of race suicide.

And lastly, should my suggestion of the granting of a State certificate of marriage be adopted, we should have the satisfaction of knowing that in future our profession would be the means of helping to compensate for loss of numbers by ensuring for the State the advent of healthy children—the best and surest evidence of a virile race.

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THE DIGESTIBILITY OF BACTERIA.

BY

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ALTHOUGH nitrogenous substances form the basis of bacterial cells, yet bacteria are not digested by powerful proteolytic ferments, and are capable of living and multiplying in a solution which would split up those proteins of which, by chemical analysis, bacteria have been shown to be composed. That this resistant faculty is not dependent on the fact of life is demonstrated by the experience that bacteria are also not digested when killed by weak antiseptics or at low temperatures. Owing to this property bacteria are enabled to continue their existence when surrounded by the digestive juices of the intestinal canal, and also continue to thrive in a culture medium into which enzymes have been excreted by other bacteria.

Kantorowicz¹ noted that after heating at 70° C. Gram-negative bacilli are less resistant to digestion than Gram-positive bacilli, and he assumed the presence of an intracellular antiprotease which holds the proteolytic activity in check. Kruse² attributed differences in survival after heating to differences in permeability of the organisms. Jobling and Peterson³ are of the opinion that bacteria resist digestion because of the lipoids with which they are surrounded, and that this resistance is proportional to the content of unsaturated lipoids.

The following experiments were undertaken for the purpose of studying the conditions under which bacteria resist digestion, and under what conditions this protective property can be destroyed. The first stage of enzyme action is considered to be an adsorption of the enzyme on to the free surface of the substrate. The question as to whether or not bacteria are capable of adsorbing ferments was therefore first studied.

Adsorption Experiments.

It is well known that bacteria are capable of adsorbing both opsonins and agglutinins from the serum, but this action is specific and cannot be regarded as an example of simple adsorption. Porter⁴ claims that membranes such as collodion sacs and egg-white films are capable not only of adsorbing but also of inactivating ferments, and suggests that living bacteria may protect themselves by the same neutralizing process.

Technique.

To test for adsorption a technique similar to that used in the agglutination saturation test was devised—namely, a dilute solution of the ferment was saturated with a heavy dose of bacteria, the tube centrifuged to deposit the bacterial mass, and the titre of the supernatant fluid compared with a similar ferment solution which had not received a saturating dose of bacteria. The ferment employed was trypsin (Burroughs, Wellcome and Co.'s "injectio trypsinii" and Fairchild's "zymine"), and the titre tested by the digestion of 4 per cent. gelatin by Sorensen's method.⁵ In this the degree of digestion is measured by the quantity of amino-acids formed. These are estimated from the carboxyl group liberated after treatment with 33 per cent. formalin solution, the acidity being determined by titration with N/100 NaOH. The twenty-four hours' bacterial growth from thirty large agar plates was washed off with saline and centrifuged, and again washed with saline. The bacterial deposit was suspended in as little fluid as possible, usually about 50 c.cm.

Thirty tubes were employed in each test. The first ten were used for determining the strength of the trypsin solution, and into each of them was placed 1 c.cm. of saline, followed by graded quantities of trypsin solution varying from 0.1 in the first tube to 1.0 c.cm. in the tenth tube, the total quantity of fluid in each tube being made up to 2 c.cm. with saline. The second series of ten tubes contained the same ingredients except that instead of 1 c.cm. of saline there was first placed in each tube 1 c.cm. of bacterial suspension. In the third series of ten tubes the trypsin acted on a suspension of fibrin or animal charcoal, which was used as a control to show the effect of adsorption. In some experiments the bacteria were left in contact with the ferment for only a few minutes, in others for as long as twenty-four hours. But the period of contact made no difference, nor did it matter whether the mixtures were left at room temperature or in the incubator. All the thirty tubes were centrifuged at high speed until the supernatant fluid was clear. One c.cm. of the supernatant fluid from each tube was now transferred to a sterile test tube containing 10 c.cm. of 4 per cent. gelatin, and placed in the water bath at 37° C. After thorough mixing a sample of 5 c.cm. was withdrawn from each tube and tested as above described. Digestion of the remainder was allowed to proceed, and another sample of 5 c.cm. gelatin removed after a definite period of time. The quantity of N/100 NaOH required to neutralize the first sample subtracted from the amount required to neutralize the second sample indicated the extent of digestion of the gelatin. A comparison of the figure obtained in each tube of the second and third series of tubes with that obtained in the first series of ten tubes indicated the loss sustained by the ferment after having been saturated by bacteria or fibrin or charcoal.

Several organisms were used, but the majority of the tests were made with *B. typhosus*, *B. coli*, and *sarcinae*. It was found that living bacteria did not adsorb trypsin at all, a result which is shown diagrammatically in Fig. 1, in which the quantities of trypsin employed are plotted against the titration readings. On the other hand, charcoal and fibrin adsorbed trypsin readily, the degree of adsorption being more pronounced in dilute than in stronger solutions. A series of experiments were made to see if organisms which had been in contact with specific immune serum could adsorb ferments. Typhoid bacilli, sensitized with antityphoid serum, were used, but were found to have no effect on ferment titre. Bacterial suspensions were made of organisms which had been boiled, autoclaved, killed with antiseptics, and others defatted with acetone, alcohol, and ether. In no case was any adsorption process observed when the supernatant fluid was tested on gelatin. It was, however, found in the case of bacteria extracted with acetone, or after boiling or autoclaving, that the trypsin after saturation, although showing no adsorption when tested on gelatin, yet showed a reduction in titre when tested on a substrate of acetone-extracted or boiled bacteria.

As will be pointed out subsequently, treatment with acetone and the process of heating renders bacteria capable of digestion by trypsin. It would seem, therefore, that adsorption does take place when bacteria are so treated as to be rendered

digestible by trypsin, but to demonstrate this adsorption it is necessary to employ digestible bacteria as substrate. This may be due to the fact that another fraction of the trypsin enzyme is required to attack the digestible bacteria from that required for gelatin. There are many who hold that trypsin is a compound of many proteolytic ferments.

It is perfectly clear, however, that bacteria in their natural condition do not adsorb the ferments with which they are in contact as do other foreign particles.

FIG. 1.—Showing effect on titre of trypsin by saturating with bacteria and charcoal.

Digestion Experiments.

Although unaltered bacteria are resistant to trypsin, yet by the application of certain chemical substances or physical processes this normal resistance may be overcome. To study the digestion of bacteria directly various methods may be employed. The simplest is to look for clearing in a turbid suspension, but this procedure is open to the objection that it does not distinguish between bacteriolysis and proteolysis, two very different processes; nor does it lend itself to exact measurement. In the experiments about to be described bacteria were digested with trypsin, and the degree of digestion estimated by the same method as that already described under adsorption experiments—namely, the rate of formation of amino-acids as the result of the digestion of the bacterial mass.

A large mass of bacteria was obtained from agar plates, the organisms centrifuged and washed and distributed in sterile tubes. Trypsin was added, a sample of the mixture removed for immediate testing, and the remainder examined after definite periods in the water bath at 37° C. Living bacteria and bacteria killed by heating for an hour at 60° C. showed no digestion whatsoever.

Effect of Higher Temperatures.—As a result of being autoclaved at 115° C. for twenty minutes all the organisms experimented with were found to be rendered capable of digestion by trypsin. Boiling for ten minutes had very little effect on the Gram-positive cocci, but the Gram-negative bacilli, *B. typhosus*, *B. coli*, and *B. pyocyaneus* were rendered easily digestible by this process. A temperature of 70° C. applied for thirty minutes was sufficient to destroy the ferment resistance of *B. coli* and *B. typhosus*.

Effect of Treatment with Acids and Alkalis.—To test the effect of alkalis and acids on bacterial antitrypsin NaOH or HCl was added to the bacterial suspension distributed in test tubes, so as to make the resultant fluids of a strength of N/2, N/4, N/8, N/16, N/32, N/64. After five minutes' contact the acid solutions were neutralized with alkali and vice versa. The tubes were allowed to stand for half an hour and then tested with trypsin. It was found that this short contact with acid had no effect on bacterial antitrypsin, whereas alkali of higher concentrations than N/10 com-

pletely destroyed the resistance of bacteria, and concentrations as low as N/30 alkali resulted in some digestion. In such experiments it is necessary to allow the alkali only to act for a short period of time because, although clearing of the solution occurs when bacteria remain in contact for a long time with weak solutions of alkali, alkali albumin is slowly formed, and this substance is not digested by trypsin. Not all bacteria behave alike when treated with alkaline solutions. Gram-positive organisms are much more resistant to alkalis than are Gram-negative.

Effect of Treatment with Fat Solvents.—Contact with acetone, alcohol, chloroform, or ether completely destroys the resistance of bacteria to trypsin. The bacterial mass may be extracted with the fat solvent in a Soxhlet apparatus, or the same result may be achieved by precipitation from an aqueous solution and allowing the bacteria to remain in contact with the fat solvent for twenty-four hours. Amino-acids are rapidly produced by digesting the dried bacterial mass with trypsin. The rate of digestion of such organisms is shown in Fig. 2.

Effect of Drying.—The bacterial suspension obtained from washing off the growth from sixty agar plates was dried *in vacuo*, and the dry powder obtained resuspended in saline and digested with trypsin. Partial proteolysis was found to take place. Similar experiments were made in which, after drying, the powder was divided into two equal parts. One half was digested with trypsin without further treatment; the other half was treated with absolute alcohol at -14° C. and dry ether at -8° C. for the purpose of extracting the fats without coagulating the protein. This was the method adopted by Teale and Bach⁶ in their work on serum antitrypsin. This extraction of the fats made no difference to the extent of the subsequent digestion.

Effect of Sensitization, etc.—A number of experiments were now undertaken for the purpose of determining how it is that bacteria are split up in the body, either when injected subcutaneously or intravenously. Since the results were all negative it will be sufficient to record them briefly. Bacteria sensitized with specific agglutinating serum were not split up by proteolytic ferments. Washed agglutinated *B. coli* treated with fresh complement were not digested by trypsin. Acetone-extracted typhoid bacilli were agglutinated with antityphoid serum, and after centrifuging the supernatant fluid was tested and it was found that practically all the agglutinins had been removed. Such sensitized acetone-

FIG. 2.—Showing digestion of *B. typhosus* by trypsin.

extracted typhoid bacilli were not digested any more rapidly than unsensitized acetone-extracted typhoid bacilli. Finally, a quantity of sterile inflammatory lymph was obtained by injecting 5 c.cm. peptone (5 per cent.) into the peritoneum of a rabbit and after eight hours washing out the peritoneal cavity. The leucocytes were removed by centrifuging. Prolonged contact with this sterile inflammatory exudate made no difference to the digestibility of the organisms employed.

Discussion.

The facts above recorded are of interest from two points of view. In the first place it is seen that those agencies which most readily destroy the bacterial antitrypsin are all fat solvents—namely, alcohol, acetone, ether, chloroform, and

alkalis. The lipoids contained by bacteria, because of their effect on surface tension, are probably concentrated round the periphery of the bacterial cells, and form a protective covering to the organism. This fatty envelope protects them from proteolytic ferments, but once this has been removed active proteolysis takes place. High temperatures and drying cause a redistribution of the lipid-protein complex, and thus, for physical reasons, disturb the protective mechanism. Therefore the antitryptic property of bacteria depends on totally different factors from the antitrypsin of egg white and blood serum.

Teale and Bach⁷ showed that the antitryptic power of egg white was due to the presence of a non-diffusible protein substance which is not affected in the dry state by treatment with lipid solvents. The same observers showed that the antitrypsin of blood serum was not dependent on the lipoids present.

Although lipid solvents appear to destroy the antitrypsin of blood serum, they do this by coagulating the proteins. Drying had no effect on the antitrypsin of blood serum, nor was it destroyed by lipid solvents when precautions were taken to prevent protein coagulation.

The greater resistance of Gram-positive bacilli to lipid solvents and heat is also capable of explanation on the basis of the physical dispersion of the lipoids.

Eisenberg⁸ found that staphylococci became Gram-negative after extraction with ether and that *B. coli* could be made Gram-positive on treatment with lecithin. Benians⁹ observed that crushed Gram-positive organisms are promptly decolorized, and concluded that the condition of the cell wall is the factor determining resistance to Gram's stain.

In Gram-positive organisms it would appear, therefore, that the lipoids are more intimately bound to the protein of the bacterial cell, thus rendering them more resistant to lipid solvents.

In the second place, it is important to note that exposure to specific agglutinating serum or inflammatory lymph does not alter the protective mechanism of bacteria against proteolytic ferments. It is claimed by Jobling and Peterson¹⁰ that bacteria treated with immune serum and complement are so altered as to be made more digestible by trypsin. They state later that bacteria treated with an excess of immune serum and complement become more resistant to proteolysis. In my experiments with *B. typhosus*, *B. coli*, and *B. pyocyaneus* it was not found possible to render these organisms more digestible by trypsin through sensitization and complement action, although many attempts were made by increasing and decreasing the sensitization and varying the quantity of complement.

Conclusions.

1. Bacteria do not adsorb proteolytic ferments.
2. The protein of bacteria is protected from the action of proteolytic ferments by the lipoidal envelope with which they are surrounded. The agencies which destroy this protective mechanism act by disturbing the lipid distribution.
3. The antibodies present in immune serum do not in any way render bacteria more digestible by trypsin.

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THE third International Neurological Congress will be held at the Salpêtrière Hospital, Paris, on June 2nd and 3rd.

THE City of Paris has authorized an expenditure of 2,500,000 francs for the purchase of radium to be used in the public hospitals for the treatment of cancer.

THE seventh French Congress of Medical Jurisprudence will be held in Paris from May 29th to 31st under the presidency of Dr. Thibierge, when the following subjects will be discussed: (1) Laboratories of criminal anthropology and psychiatric departments in Belgian prisons, introduced by Professor Héger-Gilbert and Dr. Vervaeck of Brussels. (2) The spectroscopic in medical jurisprudence, introduced by Professor Zanger of Zurich and Dr. de Laet of Brussels. (3) Bone and joint tuberculosis and workmen's accidents, introduced by Dr. Robireau. Further information can be obtained from Professor Étienne Martin, 10, Rue du Plat, Lyons.

SLIPPING RIB.

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ABNORMAL mobility of the lower intercostal joints, causing painful symptoms, is probably not a rare condition, and it is curious that it should receive no mention in the modern textbooks of surgery—at least, I have failed to discover any reference to it in the numerous volumes that I have consulted. It is for this reason that I venture to report the following two cases which have come under my observation during the past year.

Slipping rib, as it may be called, is a trivial enough complaint in itself, but it gives rise to the most irksome symptoms, and my two patients were quite incapacitated by the excruciating pain which followed any attempts to do manual work. The pain is described as of a sharp, stabbing nature, giving place after a few moments to a dull ache, which lasts for a considerable time, and may, indeed, persist throughout the day, only disappearing after the night's rest. In its position at the costal margin it resembles that due to so many deeper lesions, both of the abdomen and the thorax, that I think it is quite likely that many cases occur in which such an apparently unimportant cause as a movable rib cartilage is unsuspected, and the diagnosis is missed. It was so in one of my cases, but the difficulty here was partly due to the fact that the patient, a woman, was known to have had definite abdominal disease in the past, and to the natural tendency to attribute subsequent symptoms to this disease: still, had I been acquainted before with slipping rib as a cause of abdominal pain I think I might have arrived at a correct diagnosis somewhat sooner than I did.

The treatment is simple, and consists in resection of the loose terminal portion of the rib cartilage. In both of my two cases this was followed by immediate and lasting relief of the symptoms.

CASE I.

A single woman, aged 42, employed as a domestic servant, was admitted into Guy's Hospital in September, 1921, for right-sided subcostal pain. This had first troubled her four years before, when she fell and bruised her side, and from that time it had been present more or less constantly, being most severe when she walked much or attempted to do heavy work. Recently any effort to use her right arm had brought on such severe pain that she was quite unable to work at all, and she said that when she lifted anything heavy one of her ribs seemed to jump out of place, and that frequently a painful swelling appeared at the lower margin of the ribs. This exact description was not elicited during the earlier stages of her illness, and the diagnosis was rendered less easy by the fact that in 1918 the appendix had been removed for a basal-celled carcinoma, which led to the suspicion that her pain was due to post-operative adhesions.

In August, 1920, when I first saw her with Dr. E. P. Poulton, we decided to explore the abdomen, but at the operation the stump of the appendix was found to be healthy, and the only abnormality discovered was prolapse of the caecum and ascending colon. I performed colopexy, but this naturally enough failed to relieve the symptoms, and it was only when, a year later, she volunteered the information about the slipping rib that the real cause of her pain became apparent. It was now obvious on palpation that the tip of the tenth rib cartilage could be freely moved upwards in front of the ninth, and that this movement produced the pain of which she had for so long been complaining. Removal of the terminal three inches of the rib cartilage effected a complete cure.

CASE II.

A girl, aged 17, complained of sudden acute pain in the left side whenever she bent forward or attempted to lift anything heavy. She stated that one of her ribs had been "out of place" for some months, and on examination it was clear that the tip of the tenth left costal cartilage slipped over the lower border of the ninth rib and jutted forward beneath the skin when she flexed her body. This slipping movement of the rib was accompanied by an acute twinge of pain, which left a dull ache under the rib margin for several minutes after the rib had fallen back into place. In this case, too, removal of the terminal portion of the cartilage was followed by complete relief of the symptoms.

Very definite conclusions cannot be drawn from two cases, but it is noteworthy that both of the patients were of the female sex, and that one gave a definite history of an injury as the starting point of her symptoms. In both the pain was referred to the exact position of the affected rib, the undue mobility of which could easily be demonstrated, and in both the symptoms tended to increase as time went on.