both easier and more reliable than skiascopy which has been recently proposed. The method which I have elaborated, and practised for upwards of two years, aims at materially reducing that roll of latency. Some cases must continue to remain latent in the true sense; but it succeeds in demonstrating in a considerable percentage the presence of the abnormality, though not necessarily its nature. Moreover, it also aims at testing by means of a "normal standard" the healthy subjects who may have been passed as apparently quite free from physical signs as well as symptoms, as to the genuine soundness of their retrocaecal district. There for the present the matter must rest, as I am submitting to the Surgical Section at Liverpool the results of my clinical observations, in the hope that in spite of the onus of novelty, of any taint of false doctrine, and of the inevitable imperfections of the thing "in worden" which sometimes may hold more of the future than it quite knows, they may obtain a surgical hearing and a demonstration.

REFERENCE.
1 *Wien med. Klin.*, No. 39, 1911.

NEW METHODS FOR THE CULTURE OF BACTERIA.

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STARTING with the idea that the different tissues of the body possess their own specific salt content apart from having a specific protein content, knowing further that in each individual tissue the essential difference would be one depending on the nuclear metabolism of that tissue, recourse was had to the following procedure, which has been in use by me for nearly one year.

In every case the different organs, such as the brain or liver or skin, are freed as much as possible from blood with the view of eliminating bactericidal principles. The organs are then taken through a sterilized mincing machine and the minced material is placed in an Erlenmeyer flask, the weight of which is known. To every gram of the organ, taken originally, 2 c.cm. of sterile distilled water are added, and finally about 10 c.cm. of chloroform for each 100 grams of the mixture of the organ and water. No definite amount of chloroform can be stated, for certain organs, such as the brain, will bind more chloroform because they contain a higher percentage of fatty substances than will other organs such as the spleen in which the fat content is practically nil.

After shaking up the minced organ with the water and the chloroform for ten minutes, and then waiting for ten minutes to allow the excess of chloroform to separate out, at least 5 c.cm. of pure chloroform should be at the bottom of the flask. The Erlenmeyer flask is closed with a well-fitting stopper, perforated by a glass tube having 1 mm. diameter to allow of pressure adjustments, and is then incubated at 3 ° C. It is shaken up twice the first day and at least once every succeeding day to make sure of the whole content of the flask being saturated with chloroform. After a period varying from twenty-four hours to sixty days the supernatant fluid containing the products of digestion is poured off, and is filtered through a sterile barium sulphate filter paper (using preferably a suction pump) to get as clear a solution as possible.

The filtrate, smelling strongly of chloroform, has next passed through a stream of sterile air. Originally the air was rendered sterile by allowing it to pass through four successive Erlenmeyer flasks, of which the first contained strong sulphuric acid, the next 5 per cent. potassium permanganate, the next 5 per cent. caustic potash, and the last flask distilled water. Now I use simply a glass tube, a foot long, having an internal diameter of 1 c.cm., and filled with absorbent wool, till sucking air through the tube, by placing it in one's mouth, becomes rather difficult. This tube is slightly drawn out at the ends, and is sterilized in an autoclave along with the Berkefeld filtering plant. The candles generally used are No. N, and should never be relied on till they have been tested with bacterial cultures.

A great difference will be found in the behaviour of extracts made from different organs, even if they have

been digested for the same length of time, on sucking air through them, because some will foam a great deal more than will others—for example, the lung (because of the mucus) and embryonic skins are especially difficult to free from chloroform. It will be found best to place the filtered organ extracts into two litre flasks, and to keep the flasks constantly on the move. Apart from organs which contain mucus, which latter is not digested, or which at least does not cease to remain slimy, in all organs the foam formation will be lessened by the length of the digestion, as digestion liberates electrically active amino-acids, which, of course, lower the surface tension. Generally speaking, passing air for one to one and a half hours through 200 c.cm. of organ extract will remove the chloroform completely; but in the case of extracts of the central nervous system it may be necessary to keep the extract at a temperature of 38° C., but not higher. One of the advantages of the method given above is that each tissue has its own specific salt content—by which I mean that some tissues are rich in potash salts, while others are abundant in sodium salts, etc.

What seems at first a disadvantage, namely, the dilution of the salt content from the normal to one-third, by taking two parts of water to one of the organ, is in reality an advantage, because the hypo-isotonicity produced by the addition of water is counterbalanced by the increase in the number of molecules set free by digestion. Only in the case of nucleo-protein extracted by water is it necessary to add salt to bring the solution to about 1 per cent. isotonicity in the preliminary experiments, and then subsequently to dilute or increase the tonicity according to the effect we desire to produce in the morphology of our bacteria or other parasites. In the case of nucleo-proteins another precaution will have to be taken—namely, that of neutralization. I use a modified Locke's solution:

Dissolve NaCl=9; KCl=0.5; NaHCO₃=0.25; CaCl₂=0.25 in water 100 c.cm. Sterilize in autoclave, and add one part of this mixture to two parts of the organ extract before filtering the latter.

In the case of auto-digested organs, apart from the nucleo-protein problem, the best results I have obtained so far by using one part of the organ extract—for example, brain or liver—to from one to three parts of Deetjen's solution:

Deetjen's solution for blood platelets, leaving out the 1 per cent. agar, contains NaCl=0.6 g., sodium meta-phosphate (NaPO₃)=0.6 g., di-potassium phosphate (K₂HPO₄)=0.3 g. Water up to 100 c.cm. Boil till the meta-phosphate is dissolved. I make a stock solution ten times the strength just given and autoclave it.

The time the organs are allowed to auto-digest depends on what special radicals in the tissues we want to study in relation to their effect on bacterial growth. Thus the nucleo-proteins are readily extracted from the cells whenever the fluid medium which was used for this purpose is about one-third of the original isotonicity of the cell. The life of the nucleo-proteins in watery media in the presence of chloroform is, however, not very long, judging by the oxidase reaction of the nucleo-proteins, and therefore it is necessary to use extracts of organs which have not digested for more than twenty-four to forty-eight hours if one wants to study the effects of the nucleo-proteins on bacteria or other parasites.

Of course, if it is a question of simply studying the effect of nucleo-proteins themselves apart from auto-digestion, we would keep the material which we subsequently wish to auto-digest in an ice-chest to allow of the diffusion outward of the nucleo-proteins, then we would decant the supernatant fluid. Now we may add chloroform in the percentage stated above, and allow auto-digestion to go on in the incubator.

If we do not desire to study simply the effect of the nucleo-proteins, but also that of the cytoplasmic radicals, such as mono-amino and di-amino acids both in open-chain and ring compounds, it is not difficult to see that the longer we allow cells to auto-digest the less will the final products of digestion resemble the original combination in which these radicals existed, although the numerical proportion between the individual radicals out of which the cytoplasm was built up will not be altered.

It follows, therefore, that if it is our desire to study how different organs, after a preliminary auto-digestion, will behave towards any known bacterium or other parasite, it

is necessary to shorten the period of auto-digestion as much as possible. The same reasoning, of course, applies if we are in search of an unknown virus, which, however, produces a specific effect on a given organ, as does, for example, hydrophobia on the nervous system, vaccinia on the skin, and the lymph beneath the skin, etc.

In dealing with the immunity factor I was led by the consideration that we have to distinguish between the acquired immunity of the individual and the inherited immunity of the genus or species to which any given individual belongs. Therefore I used, in addition to the tissues and organs of adult individuals, those obtained from embryos and fetuses at different times of life, using especially cow and pig embryos, because these are readily obtained from the slaughterhouse.

The younger an embryo or fetus the less will the acquired immunity be; and the greater the difference in the species—for example, dog and rabbit—the more likely is it that we shall succeed in cultivating dog parasites in auto-digested dog extracts, and rabbit parasites in auto-digested rabbit extracts.

The above mentioned line of research has been applied by me to an investigation of the virus of hydrophobia and of vaccinia, and a detailed account will be published soon.

My reason for having suggested over two years ago to Professor Duval the use of the banana was this—that the skin and adjacent layers of the banana are fairly rich in oxidase, which latter in the presence of oxygen will act as an oxidizing medium, and in the absence of oxygen as a reducing agent. Other media which have been used by me are yellow lentils, from which the oxidases are readily extracted by 5 per cent. sodium chloride; and spleens, from which oxidases may also be obtained in large quantities by ammonium or sodium chloride.

My reasons for suggesting to Professor Duval, in connexion with his leprosy work, the three amino-acids—namely, tryptophane, lysin, and cystin—are these: Henriques showed¹ the absolute necessity of tryptophane for obtaining nitrogen equilibrium, and my own work showed the difference between acid hydrolysis and pancreatic digestion to be simply due to the fact that the former destroys tryptophane, while hydrolysis with alkalis does not destroy tryptophane, and therefore acts as do trypsin and bacteria in general. Lysin is also absolutely essential because it is absent in zein, gliadine, and mucelin, which makes these foods unable to maintain nitrogen equilibrium, as is well known. Cystin was suggested for two reasons—first, because I thought it might be used for binding deleterious by-products from such aromatic compounds as tryptophane, phenols, etc.; and secondly, because at that time I was under the erroneous impression that the leprosy bacilli attacked epithelial cells as well as the subjacent connective tissues, and, as is well known, epithelial tissues are specially rich in cystin. That both tryptophane and cystin may act as reducing agents is explained in my *Chemistry of Proteids*, where also the early history of the use of the amino-acids for bacterial cultures is discussed.

REFERENCE.

¹ *Zeitsch. f. physiol. Chem.*, 44, 466 (1907).

THE French Minister of Marine has granted permission for the use of antityphoid vaccine in the French navy, provided it is entirely optional.

AMONG numerous bequests contained in the will of the late Sir Julius Wernher are several which aim at the promotion of the study of science or at the support of charities of a medical order. Among the former is a gift of £100,000 to the Imperial College of Science and Technology at South Kensington. This institution is also given two-twelfths of the residuary estate, but such extra share is not to exceed £50,000. In regard to medical charities £20,000 is left to the German Hospital at Dalston; £25,000 to King Edward's Hospital Fund; £2,500 to the hospital at Kimberley, South Africa; £5,000 to the London Hospital; £2,500 to the Butte Hospital at Luton; and £1,000 to the Children's Home in the same town. In addition to its direct bequest, King Edward's Hospital Fund is granted one-twelfth of the residuary estate, but the sum accruing thereby is to be treated as part of the capital of the said fund and not as part of its annual receipts. Another important bequest is one of £250,000 for the purpose of assisting in the building, and if sufficient, in partly endowing, a university at Groote Schuur, near Capetown, provided that the constitution of the said university shall be approved in writing by Sir Leander Starr Jameson and Sir Lionel Phillips.

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REPORTS TO THE THERAPEUTIC SUBCOMMITTEE.

THE BACTERICIDAL ACTION OF THE CRESOLS AND ALLIED BODIES AND THE BEST MEANS OF EMPLOYING THEM.

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(Concluded from page 1297.)

V.—ON THE CHOICE OF EMULSIFICANTS FOR THE HIGHER TAR ACIDS.

SOFT soap was found to be an inefficient emulsificant for the higher tar acids. A search was therefore made for substances superior to soft soap in emulsifying power. They were at first looked for amongst proteins and carbohydrates.

Aqueous solutions of egg-albumen were useless for the emulsification of the phenols, because the protein was immediately coagulated.

Casein, on the other hand, was not precipitated by the phenols and possessed considerable emulsifying power. A 6 per cent. solution of casein was used for emulsification and was prepared by dissolving precipitated casein in a 1.5 per cent. aqueous solution of sodium carbonate with the aid of heat.

Equal volumes of the casein solutions and of the tar acids were triturated together and the tar acids were thereby emulsified, forming a white emulsion. These emulsions, however, were not readily miscible with water and they were also less permanent than emulsions of the same tar acids with soft soap.

Saturated aqueous solutions of glucose, solutions of agar-agar, and 1 per cent. solutions of starch possessed no emulsifying properties towards the higher tar-acids.

Mucilages of gum acacia and gum ghatti, however, were able to emulsify the higher phenols, but the resulting emulsions, although readily miscible with water, were not so permanent as those made with soft soap.

The emulsifying agents of protein and carbohydrate nature employed were thus not as efficient as soft soap, and a further examination of the soap class of emulsificant was made. Resin soap, although it was used as an emulsificant of the cresols, could not permanently emulsify the higher phenols. It had a further practical disadvantage, inasmuch as admixtures of resin soap and the high boiling point phenols were of an excessively viscous nature.

Castor-oil soap, however, was found to be an efficient emulsificant for the higher tar acids. Castor oil and tar acid were mixed and heated in the water-bath; the potash dissolved in water was added to the hot mixture and heating was continued until saponification was complete, this stage being reached when there was no longer a fatty scum produced when a drop of the mixture was diluted with water. The products were dark-coloured fluids, which were not very viscous and which gave stable emulsions with water.

VI.—GERMICIDAL POWER AND COST IN THE CASE OF THE COAL-TAR DISINFECTANTS.

The costs of the various preparations are best compared in terms of their carbolic coefficients. The costs of the amounts of the disinfectants equivalent in germicidal power to 10 lbs. of carbolic acid have been determined. These are obtained by dividing the price per 10 lbs. by the carbolic coefficient.

Soft soap quality P.C., which is 17s. per cwt. or about 2d. per lb., is much cheaper than the B.P. quality. As will be shown in Chapter VII., there is no advantage in using the more expensive quality and it has therefore been assumed in calculating the costs that the former would be employed.

For the purpose of calculation the following costs of other