Physiological Standardisation.

THE use of substances in the treatment of disease has, probably in a majority of cases, been empirical for some time after their discovery and adoption; in fact, in spite of recent advances in chemistry, through which the actual structure of many drugs has been elucidated, pharmacology is in many instances only able to describe the actions of drugs on living tissues without at present finding it possible to give the actual reasons for these actions. The problem is part of the wider one of the relation between chemical structure and physiological action. In those cases where the chemical structure is known, it is easy to investigate the action of known quantities of the drug upon a number of organisms of the same or different species and thus arrive at the minimum dose which is effective, and at the maximum dose which is safe, for no drug is absolutely harmless when the dose has exceeded a certain specific limit. But when a substance cannot be isolated in a pure state and its chemical structure is unknown, as is the case for example with the active principles obtained by extracting certain glandular organs, the sole indication of their presence being the effects they produce upon living tissues, it is essential, to get comparable results with different samples and to protect the patient against a possible overdose, to have an approximate idea of the strength of the sample in terms of its physiological action. It is a common experience to find that extracts prepared in the same manner and containing a known amount by weight of the original organ, may yet differ enormously in physiological activity. This variability is due to variations in the condition of the gland before extraction and to varying loss of the active principle during this process.

To illustrate the methods used in physiological standardisation, those employed in assaying the active principle or hormone of the posterior lobe of the pituitary gland, commonly known as pituitrin, and that obtained from the pancreas, called insulin, may be described briefly, with special reference to recent work on the subject.

Pituitrin acts as a stimulant to smooth muscle, affecting the muscle fibres directly; its main effect on intravenous injection is to raise the blood-pressure, but after administration per os, or intramuscularly, its absorption is too slow to produce this effect, and yet it will cause contraction of the uterus, an organ the contractile power of which is due to the smooth muscle in its wall. In clinical medicine it is used chiefly for the latter purpose in the later stages of labour. Methods of assay have been based on its effects on the blood-pressure and on the uterus. Dale and Burn (Medical Research Council Report, No. 69) have given a detailed account of the latter: the uterus of the virgin guinea-pig is used as the test object, and an extract of the posterior lobe of the pituitary, prepared in a certain manner, as the standard. It would be convenient to use as standard a substance of known composition which could be obtained in a pure condition, having the same stimulant action on smooth muscle as pituitrin. Unfortunately, tests with histamine and potassium chloride showed that uteri varied in sensitivity to these substances and to pituitrin independently.

Hogben, Schlapp, and Macdonald (*Quart. J. Exp. Physiol.*, 1924, vol. 14, p. 301) have recently described a method of assay based on the rise of blood-pressure produced by intravenous injection. It is not yet certain that the principles producing rise of blood-pressure and uterine contraction are the same, but a trustworthy method of assay of the former should be useful. Hitherto, the difficulty has been that successive doses of pituitrin produce a diminishing rise, or even a fall, of blood-pressure, and therefore samples cannot be compared with certainty. In part this is due to the presence in the extract of a substance causing a fall of pressure; this can be removed by alcoholic extraction of the gland extract, or its formation prevented by placing the gland in cold acetone, as soon as it is removed from the body. Apart from this, however, frequent injections of a depressor-free extract produce diminishing effects until a complete immunity is obtained. This can be obviated by spacing the injections at wider intervals. The test object recommended is the spinal cat, and the extract must be depressor-free, since it is possible that the substance which causes a fall of pressure in the anæsthetised animal causes a small rise in the spinal preparation. If a dose which is about half the maximum, and produces a rise of pressure of 55 mm. Hg., is injected every hour, a 10 per cent. discrimination between two samples is perfectly possible, since the effect of the previous injection has worn off in this time. The chief objection to the method appears to be in the time taken, since injections are only possible every hour; the guinea-pig's uterus, on the other hand, can receive five or six separate doses in this time. A further difficulty is that the preparation may vary slightly in sensitiveness during the course of an experiment, but this may be overcome by comparing the unknown only against adjacent injections of the standard.

The standardisation of insulin is an even more complicated problem. Its effect is to enable the tissues to utilise more carbohydrate both in health and in diabetes, when the pancreas is diseased; the simplest observation to make which shows this increased utilisation is an examination of the sugar of the blood, which falls after a dose of insulin. But if it falls too much, unpleasant symptoms, including convulsions, are produced.

Macleod and Orr (*J. Lab. Clin. Med.*, 1924, vol. 9, p. 591) have described in detail a method for assaying insulin based on the fall in blood sugar in rabbits. Owing to the variability in the response of different animals, a number must be used in any single test; the conditions must also be standardised so far as possible since the level of the blood sugar is influenced by many factors; thus the animals must be starved for the preceding 24 hours, must not have a blood sugar much above 0·1 per cent., and must not be used for more than two or three months, when they become refractory and put on weight. At least four estimations of the blood sugar are necessary in each animal, and the time occupied is considerable; hence a simpler method, if as accurate, would be preferable, and Margaret Cheadle (*Austral. J. Exp. Biol. and Med. Sci.*, 1924, vol. 1, p. 121) has utilised the incidence of convulsions as a method of assay. In this case mice are used as test animals, and minute doses of insulin injected, and the incidence of convulsions in each group injected with the same dose noted. After injection the animals must be kept at body temperature, otherwise they do not regularly develop convulsions. If a mouse unit of insulin be defined as the amount necessary to give convulsions in 60 per cent. of animals after subcutaneous injection, it must be correlated with the present standard, which is based on the fall of blood sugar in a rabbit, one unit being defined as the amount necessary to lower the blood sugar of a 2-kilo. rabbit to 0·045 per cent. in 5 hours. The unit used clinically is $\frac{1}{8}$ of the rabbit unit. The author finds 167 mouse units equivalent to 1 rabbit unit.

This somewhat cumbrous method of expressing the strength of insulin is necessary if comparable results are to be obtained in different tests with animals of varying weight. A further complication has been found, in that some samples of insulin seem to contain a substance which may be described as an anti-insulin. de Jongh (*Biochem. Jour.*, 1924, vol. 18, p. 833) gives an account of its properties: its presence is only revealed when small doses of insulin are injected, as in rabbits, but in man its influence appears to be small. It is apparently of a protein nature, and as the purification of insulin in the process of manufacture has been made more complete, the samples on the market at present appear to be free from it. In any case it is an illustration of the difficulties encountered by those who have to measure the strength of substances of unknown composition and isolated only in an impure condition.

Heterogeneous Equilibria.

THREE papers by Mr. J. A. V. Butler, in the Transactions of the Faraday Society, February 1924, and two in the *Phil. Mag.*, October and November, deal with problems of equilibrium at the boundaries between solids and liquids, and between two solids. The methods of statistical mechanics are applied in each case, and the first paper, "Conditions at the Boundary Surface of Crystalline Solids and Liquids," well illustrates the way in which other problems are dealt with.

A diagram shows how the attractions of the solid and of the liquid on a molecule of the solute and their resultant are assumed to vary with distance from the surface, with a balance point at which the two opposing forces are equal. A molecule from the surface will escape if, owing to thermal agitation, it has sufficient kinetic energy to carry it past the balance point. Molecules which reach the balance point from the interior of the liquid are attracted to the surface. An equation is deduced, similar to that of Langmuir, for the number of molecules reaching the boundary surface of a gas with kinetic energy greater than a certain quantity λ. This equation, which contains the mean collision frequency, applies to the molecules moving in the liquid towards the surface, but not directly to the molecules in the surface, the only motion of which is a vibration about an equilibrium position. The mean collision frequency is replaced in the equation for these molecules by a vibration frequency ν.

An expression for the solubility is thus obtained which leads to the le Chatelier-van 't Hoff equation for change of solubility with temperature, one of the terms of which is the heat of saturated solution. Assuming that ν is the characteristic vibration frequency of the solid, as determined by *rest strahlen*, and that the work done by the molecules from the surface layer, per gram molecule, in reaching the balance point is equal to the total heat absorbed in solution, unless this is less than the latent heat of fusion, when the latter is used, the author applies his equation to the alkaline chlorides. In this way he obtains results which are of the right order of magnitude.

Similar methods are employed in a discussion of the E.M.F. produced when a metal is dipped into a solution containing its ions. The process is regarded as essentially a solubility phenomenon. In the solution of a salt crystal, made up of positive and negative ions, both kinds are dissolved; but it is assumed that in the case of a metal, only the positive ions pass into solution, while the electrons which go to build up the crystal lattice are left behind. Equilibrium is attained when equal numbers of positive ions are dissolved from and deposited at the surface in unit time. The negative charge due to the free electrons left on the metal retards the solution and assists the deposition, and to this extent the phenomena of salt solution are modified. This is taken into account in the mathematical treatment of this case, with the result that a formula is obtained for the potential which, in form, resembles that of Nernst. Instead of being based on osmotic pressure, however, it depends on the heat absorbed in the passage of the metal ions into solution, and on quantities defining the statistical conditions. The values calculated from the formula are again of the right order of magnitude.

In a third paper Mr. Butler proposes a kinetic theory of reversible oxidation potentials at inert electrodes dipped into a solution containing two substances related by a simple oxidation reduction process. An expression is obtained for the numbers of each of the two ions, M′ and M″, contained in the solution adsorbed by each square centimetre of the electrode, using a mathematical method similar to that applied above. The reaction between each of these ions and the electrode is considered, one of them tending to gain an electron and the other to lose one, and an expression for the oxidation potential is obtained. This is determined by the ionisation potential corresponding to the loss of an electron by the reduced molecule, the difference in the energies of hydration of the two substances, the thermionic work function of the metal and two statistical constants.

Mr. Butler deals with metal contact potentials in a paper in the *Philosophical Magazine* for October. He obtains an expression for the potential difference at the surface of a single metal in a closed space containing an electron atmosphere, the loss of electrons from the surface of the metal being balanced by the gain of electrons from the atmosphere. He then considers the case of two metals in the electron atmosphere, but not in contact, and finds that though the surface P.D. of each metal depends on the electron atmosphere *concentration*, the difference for any two metals is characteristic of them. If the metals are brought into contact, the conditions at the surfaces not in contact are unaltered, and if there is to be no continuous flow across the junction, there must be a P.D. at the junction equal to the intrinsic P.D. of the metals. The Peltier heat effect at the junction is explained, and the various equations of the thermionic effect are co-ordinated. On certain assumptions they lead to the conclusion that the Thomson P.D. is the same for the same difference of temperature in all conductors.

Finally, in a paper on the seat of the electromotive force of the galvanic cell (*Phil. Mag.*, Nov.) Mr. Butler co-ordinates the results of his previous papers, and derives a statistical theory of the galvanic cell. The existence of large metal contact P.D.'s is not inconsistent with the correspondence between the E.M.F. of the cell and the energy of the chemical reaction. The metal contact P.D. theory, the chemical theory, the Nernst theory of metal electrode potential differences and the relation between E.M.F. and total energy change expressed by the Gibbs-Helmholtz equation are included in the new theory as different aspects of the whole truth.