SCIENTIFIC SECTION

RENAL FUNCTIONATION: THE PHENOLSULPHONEPHTHALEIN TEST.*

BY H. A. B. DUNNING.

Something positively non-toxic, non-irritating, rapidly and largely eliminated, quickly and easily applied; something that would give reasonably accurate showings, in the hands of the average clinician, that might be used as a renal functional test, had been anticipated for a long time and with considerable impatience by the careful surgeon and the painstaking practitioner of medicine. Various processes and substances had been tried and found more or less useful; were found to have advantages for some more specific determination, but wanting and inefficient in others, while all failed in fitness for very general application.

Now, after a world-wide investigation and trial of more than three years, it is almost universally conceded by broad-minded medical men that, for ascertaining the eliminating power of the two kidneys or the special ability of either kidney, the ideal test is closely approached by phenolsulphonephthalein.

This chemical, phenolsulphonephthalein, was first produced by Dr. Ira Remsen; later, in 1898, it was further investigated and described by one of his students, Michael Druck Sohon (see American Chemical Journal, vol. 20, No. 4, page 263), as:

A bright red crystalline powder, somewhat soluble in water, more so in alcohol, from which it was precipitated by ether as a yellowish-red crystalline powder, which on removal from the liquor instantly gave up ether, and solidified to a dark mass, which was apparently the same material.

The dilute alkaline solution is somewhat purer red than that of phenolphthalein; the more strongly alkaline solutions are purple. It is about as sensitive to alkalies and acids as phenolphthalein. The color does not disappear as readily on heating, and it might be used as an indicator with ammonia.

In a preliminary report, by the Council on Pharmacy and Chemistry, American Medical Association (see The Journal, vol. lxii, No. 4, page 298), it is described as follows:

Phenolsulphonephthalein is a bright red crystalline powder, slightly soluble in water and in alcohol with formation of a yellow solution; it is insoluble in ether. It is soluble in dilute alkalies with formation of a solution whose color is a purer red than alkaline phenolphthalein, while a strongly alkaline solution is purple. In solution it is sensitive to carbonic acid just as phenolphthalein is. It is readily soluble in sodium carbonate solution, and shows stronger acid properties than any of the related phthaleins. The substance at first has a slightly sweetish taste which changes to a disagreeable bitter taste.

The substance is marketed in the solution of its monosodium salt only; the solution (6 mg. to 1 Cc.), biologically standardized and sterile, is contained in hermetically-sealed ampoules, each ampoule holding more than enough for a test.

It was Dr. John J. Abel and his associate, Dr. L. G. Rowntree, while investigating the pharmacodynamics of all the phthaleins, some years after the publication by

* Presented to Scientific Section, A. Ph. A., San Francisco meeting.

Sohon, who discovered the remarkable manner in which normal kidneys disposed of this compound—phenolsulphonephthalein—fully 85 percent of an injected quantity being thrown off through renal functionation within two or three hours.

Doctors Abel and Rowntree, of the Pharmacological Department of the Johns Hopkins Medical School, further examined the substance pharmacologically and found it to be even less toxic than sodium chloride. See The Journal of Pharmacology and Experimental Therapeutics, vol. 1, No. 6, page 595, from which is quoted as follows:

The pharmacology of this substance has been studied by Abel and Rowntree, who have shown that properly-prepared solutions of the sodium salt can be injected under the skin without the slightest evidence of an irritant action; that the drug can be administered by mouth without untoward effects of any kind; that the administration of the drug in 0.1-gramme doses or less by mouth is followed by its appearance in the urine in one to one and a half hours; that the subcutaneous administration is followed by the appearance of the drug in the urine within ten minutes. The method of its excretion in the bile and urine and its absorption from the bile in the intestine was also investigated. Following the subcutaneous injection of 1 gramme, the drug appears in the bile in high concentration in one to two hours. It is then reabsorbed by all parts of the intestines, and only a trace, even after these large doses, can be found in the stool.

The effect of 1-gramme doses of the drug upon the kidneys of dogs was also studied, and in no case was albumin, sugar, casts, or other abnormalities found. It was found, however, that large doses given intravenously to rabbits exerted a mild diuretic action.

Further investigation yields additional evidence of the non-toxicity of this substance. One of us (R.) has tested the effect of this preparation on rats infected with various strains of trypanosomes. In one instance 0.3 gramme was administered to a 200-gramme rat and repeated without any untoward effect.

A small kitten, weight 650 grammes, was given 0.115 gramme of phenolsulphonephthalein subcutaneously. The stool showed only a trace of the drug, while the urine was red for sixty to seventy-two hours following the injection. No toxic symptoms were present.

This reference is from the published study of Drs. L. G. Rowntree and J. T. Geraghty, to whom is due the credit for first using phenolsulphonephthalein as a renal test and for first making practical and successful applications of it to clinical diagnosis. These investigators acknowledge assistance in their efforts to Doctors Abel, Young, Slagle, Barker, Thayer, McCrae, Hobelman, Merrill, Kelly, and Richardson, also to H. A. Brown Dunning, Phar.D., who made and supplied most of the phenolsulphonephthalein used in their investigations and who, through a long series of experiments, succeeded in modifying the original methods of producing phenolsulphonephthalein and, still more important, originated a method of purification which yields this substance, pure to a degree entirely satisfactory to the originators of the test.

REMARKS ON PHENOLSULPHONEPHTHALEIN.

Phenolsulphonephthalein was first prepared by Ira Remsen and Sohon by heating in hot-air bath at 130° C. for 24 hours orthosulphobenzoic acid anhydride with twice the molecular equivalent of phenol. The resulting resinous mass is thoroughly disintegrated by boiling water until free from phenol. The substance is then collected upon a filter and thoroughly washed and, finally, is transferred to porous plates and dried, or it may be dried in a current of warm air on filter-paper. While the mass has a beautiful deep-green fluorescence, which presents a

blood-red streak when the surface is broken, the washed and dried powder varies in color from a beautiful vermilion red to a brownish red, depending upon its fineness. The substance is soluble in cold water to a slight degree only, yielding a deep-yellow solution, which becomes Bordeaux red upon the addition of one molecule of sodium hydroxide, giving a beautiful purple with excess of the alkali. Similar to phenolphthalein, the aqueous solution of the sodium salt is effected by carbonic acid, but the color of the dilute solution seems to be unaltered, if kept in a tightly-stoppered vessel. The substance is soluble in alcohol, but insoluble in ether. The starting-point of Remsen and Sohon was commercial saccharin, containing about 60 percent benzoic sulphinide. It may be of interest, at this point, to state that commercial saccharin, at the present time, is practically pure benzoic sulphinide.

Following the method of Remsen and Saunders, the saccharin in aqueous mixture was boiled with hydrochloric acid until solution of the benzoic sulphinide was effected and converted into the acid ammonium salt of orthosulphobenzoic acid, which is evidenced by the disappearance of the sweet saccharin taste. The undissolved matter is parasulphaminebenzoic acid, from which the acid ammonium salt in solution is separated by filtration, subsequent to concentration and cooling of the mixture. The acid ammonium salt was decomposed with potassium hydroxide in hot concentrated solution, thereby forming the potassium salt. The acid potassium salt was precipitated from the cool solution by the addition of concentrated hydrochloric acid. The washed and dried potassium salt was then treated with phosphorus pentachloride and the cool mass extracted with benzene, which brings into solution the anhydride of orthosulphobenzoic acid.

The method adopted by Slagle, who prepared the " phthalein " at the Johns Hopkins Medical School for experimental pharmacological purposes, is, in a large measure, selected from the work of Remsen and Sohon, Remsen and Saunders, and Remsen and Dohme. Slagle started with saccharin and produced the acid ammonium salt of orthosulphobenzoic acid in the way mentioned above, then treated the acid ammonium salt with phosphorus pentachloride, producing the chloride of the acid, which is a yellow, oily liquid of high specific gravity, possessing a peculiar, sweetish, unpleasant mouse-like odor. After thorough washing, the chloride is boiled with water, which decomposes it, with the formation of orthosulphobenzoic acid, which, upon evaporating to dryness and heating to remove hydrochloric acid, is mixed with phosphorus pentoxide and distilled to obtain the anhydride of the acid, which distils as an oily liquid at a high temperature, quickly congealing to a white, opaque, crystalline mass in the receiver. The value of the substance for use in medicine was determined, under the direction of Dr. John J. Abel, by Dr. L. G. Rowntree, of the Pharmacological Department of the Johns Hopkins Medical School, while the clinical work was done by Dr. J. T. Geraghty, of the hospital staff. These investigators learned, after extensive experimentation, that when " phthalein " is injected subcutaneously the entire amount is excreted by the normal kidneys in a limited time: from 40 percent to 60 percent during the first hour after its first appearance in the urine, which requires about ten minutes, and about 60 percent to 80 percent within two hours. The essential features of the test are as follows:

One Cc. of the solution containing 6 milligrammes of " phthalein," as a monosodium salt, is injected into the arm or the buttocks; after about ten minutes, the " phthalein " may be detected by catheterizing and passing the urine into a test-tube containing solution of sodium hydroxide, thereafter for one hour the entire amount of urine excreted is collected. The present procedure is to collect excreted urine for one hour and ten minutes from time of giving injection. Sufficient

sodium hydroxide solution is added to make alkaline, and the whole is diluted to 1000 cubic centimetres. The quantity of " phthalein " eliminated is determined, colorimetrically, by comparing the specimen collected, as above stated, with a standard solution of " phthalein " prepared by diluting 1 Cc. of " phthalein " solution containing 6 milligrammes to 1000 Cc. with water made alkaline. This comparison is most accurately made through the use of a colorimeter. The value of the test depends upon the following facts: 40 percent to 60 percent of " phthalein " injected is eliminated by the normal kidneys within one hour, irrespective of the quantity of urine excreted. If the amount of the substance eliminated is very low, 5 percent to 30 percent, then the kidneys are not functionating properly.

This diagnostic test of renal efficiency has attracted widespread interest, not only in this country, but in England, France, Germany, Japan, and Australia.

LABORATORY OF HYNSON, WESTCOTT & COMPANY, Baltimore, Md.

PROGRESS IN THE CULTIVATION OF MEDICINAL PLANTS.*

BY HENRY KRAEMER, PH.D.

For the past fifteen years I have supported the theory that the cultivation of medicinal plants in the United States was not only practicable but essential for the scientific development of manufacturing pharmacy. As long ago as 1902 Doctor True wrote me as follows:

" This matter of the domestication and cultivation of our native drug plants is one that is interesting me a great deal and seems likely to take some of my time for the immediate future. I hope I may be able to trouble you often in connection with this attempt to open up a new line of agricultural work. I have no doubt that the right man will be able to get rich by cultivating drugs, if we can find methods of handling the articles so as to produce a first-class product."

During these years the work has been progressing very slowly and yet with very great satisfaction. Many students of botany have had their small gardens in which they grew a limited number of plants. I well recall that the late Professor John M. Maisch had a small garden in West Philadelphia, and so have others had these gardens. My first work was done in a side yard. In 1907, when the Foods and Drugs Laboratory of the Philadelphia College of Pharmacy was built, a lattice platform was laid on the top of the roof and we built a roof garden. We constructed our boxes, purchased about ten cart-loads of good soil, and went ahead planting trees, shrubs, and vines. We arranged beds with lattice for those plants that required shade. Out of an old zinc-lined trough we constructed a Jersey bog, in which iris, cat-tails, drosera, sarracenias were planted and bloomed. In the fall of 1909 the college erected a good green-house. During these years we have had under observation over 300 different species of plants. Most of these plants have been used in connection with investigations of crude drugs. In addition, the work has given us valuable data in regard to growing plants by means of seeds and cuttings, the drying and curing of them, and, in a few instances, of extracting their constituents.

It is not so much of my own experiments that I want to talk about to-night as it is the progress of the work done by others. I desire to refer to the splendid equipment of green-houses and grounds by Prof. E. L. Newcomb at the University of Minnesota. Professor Newcomb was associated with me when we

* Read before the Philadelphia Branch, A. Ph. A., December 13, 1915.