and tincture after standing this length of time still retains sufficient activity for them both to be considered standard preparations.

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ABSTRACT OF DISCUSSION ON DIGITALIS PAPERS BEFORE SCIENTIFIC SECTION, A. PH. A.

ROBERT A. HATCHER: I have a number of questions. I hope I won't seem too inquisitive. I have been very much put to it to secure my favorite animal, the cat, in testing, and I have been casting about to see if I could substitute frogs for cats. That might come as a shock to a good many members of this assembly, but it has been impossible at times to secure an adequate supply of cats. I have been experimenting on frogs, and while I had from time to time standardized various preparations on frogs, I recently purchased a batch of five hundred frogs with which I was utterly unable to standardize a single preparation of digitalis and could not even standardize the frogs against ouabain. On three hundred and seventeen out of five hundred frogs I could not standardize the frogs against ouabain. Now there are some here who will throw up their hands in horror at my inability to do this. I have, however, consulted with others, who informed me that the uniformity of absorption is the bane of the work in the hygienic laboratory; that they occasionally have similar experiences. Now if gold fish eliminate the uncertainty due to variation in susceptibility I am certainly glad because of the saving in time consumed. I want to ask if the same ratio exists between the toxicity of tincture of strophanthus and tincture of digitalis when tested on gold fish or when tested on cats or frogs?

PAUL S. PITTENGER: I had better answer the questions as they come up. We have not gone far enough with the gold fish method to find the exact ratio between digitalis and the other drugs. We intend to take up strophanthus and the other drugs later on. We have, however, started with ouabain and found that it took a very much greater amount of ouabain in comparison with the amount of digitalis required to produce toxic effects than on either frogs or guinea pigs.

ROBERT A. HATCHER: More ouabain?

PAUL S. PITTENGER: No, not more ouabain than tincture of digitalis, but many times more ouabain *in comparison with the amount of digitalis* than by either the guinea pig or frog method.

ROBERT A. HATCHER: Where it is not necessary to be absolutely accurate what are the ranges of accuracy with slight variations in temperature? It is rather difficult to maintain a temperature that does not vary over a degree.

PAUL S. PITTENGER: All these tests were carried out with a variation in the temperature bath from one to one and a half degrees, with the accuracy of the test well within two and a half percent.

HENRY KRAEMER: I was very much interested in the paper of Dr. Pittenger because I have long realized that gold fish were exceedingly sensitive to chemical substances. A few years ago a client came to me to investigate a problem. He had some trees which had been destroyed by a Public Service Corporation tearing up the streets, affecting the gas mains. So I was called in about eighteen months after the damage had been done, to act in the capacity of an expert. I went out and saw the trees were all dead. The question was, what had killed them? It occurred to me that there ought to be some definite, scientific way of showing the cause. The soil was clay, so I had a man dig down about six feet and obtained some samples. It occurred to me that probably a very simple test could be performed by comparing my samples with ordinary soil from some other locality. I bought a couple dozen gold fish and it was surprising as to how immediate the reaction was. After putting a sample of the soil, which I had obtained from the roots which were injured, in the water the gold fish immediately succumbed. One thing you have to watch in the city, where you have flowing water, is the effect of the chemicals used in treating the water. One time, for instance, I was making starch water, and was surprised to see how much the water tested of chlorine. Such things are very apt to affect some work, when you are expecting definite reactions you may have those from the chemically treated water. I merely interject this so you will appreciate that there are some things apart from the

sensitiveness of the gold fish that will have to be taken into consideration. There is no question but that gold fish are wonderfully sensitive to a poisonous substance like chlorine gas.

HERBERT C. HAMILTON: I have been very much interested in the subject of the use of gold fish for standardizing digitalis. I recall very well the presentation of Dr. Pittenger's first paper quite a number of years ago. About twelve years ago our firm was submitted a sample of some kind of root that was used as a fish poison in the East Indies or somewhere in a foreign country. It was put up to us to see whether it contained anything of therapeutic value. It seemed to be not particularly poisonous. I tried the drug on gold fish. As Dr. Pittenger and Dr. Kraemer have mentioned, I found the gold fish exceedingly sensitive to the material. While the possibility of using them for standardizing drugs never occurred to me, it certainly was a wonderful means of demonstrating the value of that fish poison. There is one question that I do not know whether Dr. Pittenger brought out, as I did not hear all his remarks. I would like to bring out the point as to whether the test could be shortened up in time, and instead of three hours have the time nearer two hours without seriously impairing its accuracy. Another point I want to speak of. Objection might be raised on the point as to whether the toxicity test is a clear indication of the therapeutic value of these heart tonics. One of the things we always think of in connection with the use of frogs, in testing, is the opportunity of examining the heart to see if it is contracted, which is very characteristic of digitalis and is a means of eliminating the frogs that die from other causes. It seems to me that this is one and may be almost the only point that can be raised against this method of testing.

PAUL S. PITTENGER: In regard to the time, I would state that the paper shows very conclusively that the results obtained with a time limit of exactly three hours are more accurate than those obtained by a shorter or longer limit. We ran experiments using doses large enough to kill in from one-half hour to two or three hours; from three to six hours; from six to eight hours; and from twelve to twenty-four hours; in other words, to see which time limit gave the most sensitive results. We found the method most sensitive by using a limit of three hours. That is the reason we adopted three hours for the time limit. Using the method described with a three hour "time limit" we were able to get accurate results down to a variation of only five hundredths of a mil of tincture of digitalis in 500 mils of water. Of course it is almost foolish to try such small quantities, but we actually did get fairly concordant results with a variation in the dosage as small as this. Although some fish died "out of order" the M. L. D. could be accurately determined. In regard to the toxicity, I have not carried out any experiments to prove whether the results obtained on fish parallel those obtained on frogs. This can very easily be determined, however, by testing a series of drugs by both methods.

Discussion on papers by L. W. Rowe and Robert A. Hatcher.

JACOB DINER: Both Dr. Rowe and Dr. Hatcher are to be complimented. I was very glad to hear Dr. Rowe bring out the inconsistency between the action upon the cat, the dog, and the frog. It merely brings home to us again the fact that the pharmacologists have been getting into a rut and the sooner they get out of that rut the better it will be for humanity. Animal experimentation has its proper place, but in my opinion animal standardization as it is being carried out today is not right. We are not interested in killing the cat or dog or frog or the gold fish. We are interested in obtaining certain therapeutic effects upon a pathologic heart, being either functional or organic. I believe that the clinicians are coming more and more to rely upon advertised articles merely because there is a claim for them of certain therapeutic value. Dr. Hatcher is to be congratulated for his very ingenious and praiseworthy efforts in regard to the action of digitalis, showing that the chloroform-soluble and the water-soluble portions are entirely distinct groups in their action, and if they are distinct in their action on the animal it is fairly reasonable to assume that they may be much more distinct in their action on the human being. I was delighted to hear that Dr. Hatcher will insist upon clinical observations. After all that is the only test. It is true that we cannot use men for standardization, but we certainly can use them for clinical observation. Upon that and that alone should we base our final answer. It is too bad that we cannot find the certain dose, but from personal observation I might state that I still use digitalis by the mouth and I frequently obtain very satisfactory results.

HERBERT C. HAMILTON: Speaking on the line that Dr. Diner has just discussed, I think there is a misconception possibly among pharmacologists, a misconception as to what standardiza-

tion and testing means. A pharmacist takes a solution containing an alkaloid and he titrates or shakes it out with chloroform or ether or something like that and his test has absolutely no relation to the therapeutic value of the drug. I have always contended that a biological test is the standardization test comparable to a chemical test, chemical or pharmaceutical, and that there is no necessity of there being any relationship between a standard test dose and the therapeutic dose. A therapeutic dose is something that must be determined by clinicians; a standardization dose may be entirely different and may bring about an entirely different set of reactions. I think, of course, if there could be a determined relationship between the two, it would be very nice, but when any one asks what is the relationship between a standard test dose for a cat, a dog, a guinea pig, a gold fish, or a human being, I think everybody would admit that it is rather absurd that there could be any relationship between them. I always have contended that the cat method was merely another of the toxicity tests with no definite point to indicate whether the death of the animal was due to digitalis. Of course, with careful work there would be no reason for suspecting anything to be present in digitalis or strophanthus that did not have direct action on the heart. Still there is always the possibility of decomposition products being present and producing the death of the animal instead of the principle of therapeutic value. The frog method always gives one the opportunity of examining the heart to see, by the position of the heart, whether the death of the frog was due to digitalis or not.

OTTO RAUBENHEIMER: This is evidently the age of learning, and Dr. Hatcher is going to revolutionize not only the therapeutics but also the chemistry of digitalis. Up to now the theories have always been that the higher the alcoholic content of the menstruum, the better the glucosidal preparations keep. This is the reason we are using ninety-five percent of alcohol for strophanthus. This is the reason we are using seventy percent alcohol for tincture of digitalis. Now Dr. Hatcher comes along and says—I hope he is right; he is always right—he says that water extracts it all. What cheaper solvent can you get than water? Why, pharmacists will all be happy making tincture of digitalis with water, and extracting all the virtues of digitalis. Dr. Hatcher is to be complimented on his experiments. He has kept on for ten years, as long as I have known him. I believe he will after all reach that conclusion which has been well established for years and years, that *wild grown* digitalis is the most active drug.

HENRY KRAEMER: Fortunately there are so many observers at the present time, and we see this subject from our experience in a good many different ways, I want to just interject a thought to Dr. Raubenheimer, that in my experience the wild grown digitalis is not by any means equal to that which is cultivated. I had an experience last summer in growing—I forget how much we did grow but it was considerable—several hundred pounds of digitalis. In fact, my plants grew so fast that I was at a loss to know whether I could bring them in or let them go to waste. All that I could do was to bring the digitalis in and let it dry as best it could, taking the *whole* plant and stringing it on wires, and so on. That digitalis is three times the strength of any digitalis on the market, as far as known.

I want to bring to your attention one thought in connection with chloroform extract. I have been working for fifteen or twenty years with these things, with chlorophyl, etc. Here is my point for you to think about. This chloroformic extract will look different if its alcoholic solution is reduced at some point with zinc. Furthermore, if that reduction product is kept from the air by inverting the bottle you will be surprised what a change it will make. Doctor, that is a very important contribution, because we will never formulate a preparation of digitalis until we know more about these constituents.

OTTO RAUBENHEIMER: I still maintain that the digitalis that Dr. Kraemer spoke about was wild, because he said he neglected its cultivation. It must have been wild.

HENRY KRAEMER: I did not neglect the cultivation. I did not pay any attention to all those modern methods that are sometimes assumed to be necessary in the harvesting of digitalis. I did not mean to infer that my own method of the preparation of digitalis was not probably more scientific than the other.

PAUL S. PITTENGER: First of all I wish to state that my observations agree with Dr. Kraemer's, namely, that cultivated digitalis as a whole is very much more active than the wild or uncultivated drug. At our Glenolden farms we have been able, through cultivation, to produce for the past several years hundreds of pounds of digitalis, containing two, three and even four times our standard activity. Next, I would like to ask Dr. Hatcher if he made any tests to

determine what proportion of the entire activity of the drug was contained in each of his two extracts and whether the activity of the one extract added to the activity of the other would equal that of an extract of the whole drug?

ROBERT A. HATCHER: Whether the separate constituents represented the entire drug?

PAUL S. PITTENGER: Yes, whether there were any comparisons made between the activity of your extracts and a tincture made from the same drug.

ROBERT A. HATCHER: In one effort to get rid of the chlorophyll I lost ninety percent of it in refining it. I did not always get the total, but it comes out this way. For this experiment I used six hundred and ten grammes of powder to make the infusion. The total mixture of all of these came out seventy-nine hundred cat units for the total six hundred and ten grammes. A total of the separate substances, I think, came out something like seventy-one hundred. I only made a few tests. The separate infusions came out seventy-nine hundred. I believe this came out one-tenth short, but that was a loss I was not particular about because in these precipitates I did not make any effort to get out the last trace.

PAUL S. PITTENGER: In reference to the cat method for general standardization purposes, I find that fairly accurate results can be obtained with this method, but our principal trouble is the same as with the other workers, namely, the difficulty in obtaining enough cats to carry out our assays. In fact, in Philadelphia it is almost impossible to get cats. I can get dogs. I send men away with dogs; not so with cats.

Now as to the time. Granted that a single test by the cat method takes less time to carry out than the frog, guinea pig or gold fish method, I do not think that that is true when you have a number of tests to carry out at the same time. We often have from fifteen to twenty samples at one time. With either the frog, guinea pig or gold fish method you can work on the whole series at one time. You can give all your frog or guinea pig injections in fifteen or twenty minutes, after which, in the case of the guinea pig method, there is nothing to do until the next day, or, with the frog method, for an hour. In this way you can work on fifteen or twenty tests at one time. With the cat method you can only work on one animal at a time, and it therefore takes a great deal more instead of less time. I think Dr. Rowe is to be congratulated on his constant temperature tank for frogs, because that is something the practical laboratory needed for quite a long time.

LYMAN F. KEBLER: I have been interested in this discussion. I confess that there still seems to be so very much that is unsatisfactory in these methods. We, of course, in our work are asked to pass on the quality and character of goods imported into the country and especially from one state to another. We have tried to use some of these physiological and biological tests, as you call them, and then run up against exactly these various propositions, and the result frequently is that the goods have to be released, and, therefore, they come on the market probably not coming up to the strength called for by manufacturer A or manufacturer B. It is very interesting to find that after you do release some of these products or drugs, manufacturer A finds them perfectly satisfactory but manufacturer B does not find them satisfactory at all. He calls them unreliable and we are criticized for letting them in. Here is the other proposition: Suppose that we have these methods and we run up against these snags—these apparently unsatisfactory conditions that our friends record here—what are we to do about it? Are we going to hold up a man's goods until these things are digested? We cannot do that. As a matter of fact, the situation now is that we have to let the goods in under the conditions that obtain. I certainly hope that something will be done to rectify the situation. Probably the criticism sometimes comes that we let in and permit shipment of low-grade adulterated goods. That may be under the conditions by which we are operating. That not only holds for these biological tests but some of the other tests that we run up against. Dr. Kraemer reported some article with fifty percent adulteration. We should take that up and see where we stand.

ROBERT A. HATCHER: May I say that my ambition is to see this done. We are really going the wrong way around about the whole method of the examination of tinctures. We are going at it in a positively silly fashion. At the outbreak of the war I was in communication with the medical branch of the War Department, and had about gotten a promise to have enough crude digitalis leaves assembled in one single lot which was to be mixed thoroughly and then tested, after which all the tincture of digitalis of the United States Army was to be made from that single lot. In this way, no matter where a physician went from camp to camp, wherever

he might be he would have the same tincture to deal with. That was carried out partially. The Government assembled six hundred pounds at Minneapolis and six thousand pints of tincture digitalis was made from that one single lot. But unfortunately it did not supply the whole Army. Previous to this on testing again and again specimens for the Army in different camps, I could find no activity, and they could not get any effect, and that was simply because the digitalis was of inferior grade.

AMBROSE HUNSBERGER: I am in sympathy with Dr. Hatcher's suggestions because the question of digitalis has been one that interested me particularly during the past year. I was also interested in Professor Kraemer's statement regarding the digitalis that he grew, comparing it with the digitalis on the market at the present time. I was wondering whether he compared it with the digitalis that he bought on the market during the past year or whether he found it two or three times as strong as the U. S. P. standard.

OBSERVATIONS ON DIGITALIS SIBIRICA.*

BY HEBER W. YOUNGKEN.

About two years ago, the writer received a number of samples of medicinal plant seeds from the Bureau of Plant Industry of the United States Department of Agriculture. Among these was a generous supply of the seeds of *Digitalis Sibirica* Lindley.

Some of these seeds were sown in a seed pan in the greenhouse of the Philadelphia College of Pharmacy. They were found for the most part viable, for ere long they germinated into a goodly number of seedlings. These seedlings were later transplanted to boxes on the roof garden containing ordinary garden soil. Here they thrived so well, forming, in most instances, a good rosette of leaves at the end of the season, that his attention became more concentrated upon them.

He next conceived the idea of determining how the pharmacodynamic properties of their leaves compared with those of *Digitalis purpurea* Linne. The results of a biologic assay, performed by testing the tincture of the leaves (prepared in accordance with the U. S. P. method prescribed for Tincture of Digitalis) on normal frogs at a temperature of 22° C. by the one-hour frog method, showed the tincture to be three-quarters over the strength required for the U. S. P. Tincture of Digitalis. Moreover, in every case where the dose was toxic, the heart was found to have stopped in systole. The last effect is the same as has been recorded for all the members of the Digitalis series of cardiac tonics.

As yet he has not had the opportunity to study the pharmacotherapy of the leaves, but, judging from the results of the biologic assay, they may later be found at least as efficient as those of *Digitalis purpurea* L.

Upon searching the literature for botanical references, only one of any value could be found. This was a short article by Lindley in "Digitalium Monographia" published in 1821, in which he pictures the plant in colors, mentions a few of its macroscopic characteristics, and states that its habitat is Siberia and Tartary.

The meagre data on this plant up to the present, linked with the facts that it is so easily grown and has strong prospects, on account of its pharmacodynamic properties, of becoming one of our valued cardiac tonics, inspired the writer to make a botanical investigation, the results of which are hereby presented.

*Read before Scientific Section, A. Ph. A., New York meeting, 1919.