possible to have them, and in this respect your chief concern should be that biological products should be kept cool—actual contact with ice is all but imperative with the most useful and important of the whole list—smallpox vaccine.

BIOLOGIC ASSAYING: ITS SCOPE AND LIMITATIONS.*

BY HERBERT C. HAMILTON.

To many of you this subject of biologic standardization may seem hackneyed and time-worn. Among my earliest recollections in connection with this subject was a controversy between the representatives of two pharmaceutical manufacturing firms as to whether it is possible to make the test quantitative, neither party questioning its truly qualitative character when properly applied.

Now, however, the question seems to have advanced a point. It is apparently doubful in some minds whether it is even qualitative. It was stated recently that "If you would know the effect of a drug on a human it must be tested on a human; this cannot be deduced with any degree of certainty by its action on one of the lower animals."

Is there any excuse for continuing an apparently profitless discussion? There is more than an excuse, there is a reason and a vital one. To each of us, either for himself or for some one near and dear to him, it is a vital question since few of us are fortunate enough to escape the physician and the druggist.

If you respond that most of the drugs we use are standardized chemically or are so harmless that they need no standardization, it is really a strong point for biologic standardization for why should any powerful agent be left to chance if a method can be applied by which a uniform product results?

Is there any less reason why the physician and the patient should be able to purchase standardized digitalis, ergot or antitoxin than for us to be able to buy standardized solutions of strychnine or morphine?

But some will say that standardization of digitalis does not insure potency when you buy it some months or years afterwards. But it does insure the marketing of a uniform product from a drug which is highly variable.

Digitalis grows under many varying conditions of climate, season and soil, sometimes cultivated, sometimes not. The time of gathering, the method and efficiency of the drying, the extraction, all may influence the activity of the final extract. Should this be left to chance if it is possible to make it a certainty?

But, you may reply by the question "How much certainty is there when the basis of the test is only that the drug will kill a dog, cat, pig, frog or gold fish?" That question, however, is really beside the point. The question of killing is unimportant; it is the amount that kills and the character of the death. If two tinctures of digitalis are tested on cats or frogs and one is found to kill with one-half the dose required for the other, which would the physician choose? Or, if two tinctures are tested on frogs and one stops the heart in systole while the other, although equally toxic, consistently leaves the heart in diastole and when tested on the laid-bare heart does not slow the rhythm, one must conclude that there is little digitalis in the solution. The latter may contain some digitalis activity

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and is certainly toxic but from causes other than the digitalis glucosides. This case is not a probable one, but it is always possible.

But you say again what connection is there between the dose that will kill a frog and the therapeutic dose? None whatever! Neither is there any connection between the amount of acid used in titrating an alkaloid and the dose of the alkaloid in any particular case. The conditions are parallel, except that in the chemical assay it is possible to have a reagent of known strength or purity while in the biologic assay the reagent—the animal—is a variable factor and must be checked up for its sensitiveness at time of assay.

A knowledge of pharmacology must precede biologic assaying. The drug must first be studied carefully on animals to determine in what respects it is most active if it has more than one typical effect. Among these effects one is to be selected as being typical and measurable, that is, showing degrees of activity dependent on the size of the dose.

But, you say, pharmacologists do not agree among themselves! They are not able to decide which is the most accurate test. That is unfortunately true. This, however, may not be a serious objection for each investigator uses the method best adapted to the equipment of his laboratory and to his mental attitude.

It is not illogical at this point to note some of the different reactions which follow the administration of drugs.

Digitalis slows and strengthens the heart beat and raises blood pressure by its action on the arterial walls. In toxic doses it causes arrhythmia, heart-block and, finally, death. In frogs the heart stops in systole with a dose somewhat less than that which causes death. At death the heart is in systole, which is typical of the digitalis series of heart tonics. Ergot both raises and lowers blood pressure because it contains principles which have opposite effects. It causes stasis of blood in the vessels. It acts on the uterus muscle, causing contraction. Cannabis sativa causes typical intoxication in dogs with incoördination, one of the most characteristic features, with lowering of temperature and drowsiness.

It is evident from the above why pharmacologists differ in their opinions as to which effect is most typical or capable of most exact measurement.

But a still more serious objection has been voiced—that one cannot conclude from the action of a drug on animals what its action will be on man.

Partly true again. But can one be certain that any drug will invariably have the same effect on one human that it had on another? Answering the objection that the test and the clinical results are unlike is best done by illustration. Antitoxin for diphtheria neutralizes the toxins of the disease. That is practically its only function. It is assayed by neutralizing a toxin. The potency of the unneutralized toxin is determined on pigs; then its decreased potency is measured after partial neutralization, known amounts of the toxin being treated with different amounts of the antitoxin until its potency is destroyed.

Pituitary extract activity is measured by its constricting action on muscular tissue, the stronger the solution the greater the constricting action. It is measured on the uterus muscles because it is used clinically to bring about contractions of this particular muscle. It is also measured on the muscles of the arteries because clinically it has application to overcome the collapse following operations. This seems to be directly dependent on the blood pressure.

Adrenalin and suprarenal extracts are not only used but also standardized as hemostatic agents. All through the list of the biologically tested drugs the same rule holds good—the method of testing runs closely parallel with some use of that drug in therapeutics. The only important difference is that while the biologic assay is based on the same reaction that gives the drug its therapeutic value, the therapeutic dose is often small in proportion to the assay doses: the latter must produce an extreme effect observable in a short time, the therapeutic dose an almost intangible immediate effect.

The logic of this objection is at fault because of confusing a quantitative assay with a qualitative or pharmacologic study of a drug. It must not be overlooked that the assay process is not a qualitative study. The pharmacology of the drug must have been studied first just as qualitative precedes quantitative analysis.

About this time some one should say "But you pharmacologists are always saying how uncertain the biologic test is because the animal may not react properly just when you want it to!" True, alas, curses come home to roost! But in extenuation of this remark it must be observed that if the one animal, or set of animals, fails to react properly, we are apt to paraphrase the expression about the "perversity of inanimate things" and try again. It should not be overlooked that tests on the human sometimes do not permit of a second trial.

But how about the chemical test? Does it never go wrong?

Some years ago Haskell, examining some tinctures of aconite, all of which were adjusted to standard by chemical assay, found that they differed greatly in activity when tested on animals. One was 10 times as strong as the weakest one. But they looked alike and the alkaloidal content was almost identical in all of them. The alkaloid, while retaining its characteristic chemical properties, had evidently suffered some change which lowered its toxicity and also its therapeutic value. Clinical results are difficult to obtain with a drug of this character. Would you still say that one cannot deduce the physiologic action from the effect on the guinea pig?

Has biologic assaying any limitations? Alas, yes! Not only do animals act erratically, sometimes responding to the drug with small doses and remaining immune to large ones in a truly human way, but we are at times obliged to ignore the most promising reaction of a drug because the effect is not measurable. For example, digitalis as a pressor agent, strophanthus, to measure its action on the heart directly, squill as an expectorant; even for the drugs commonly tested chemically we occasionally could use to advantage a measurable biologic test as a check but have no typical effect of this character.

Rusby says that many are skeptical as to whether the drug with the greatest power to kill a dog has the most value for curing a man. Thus baldly stated, the comment places biologic assay in an awkward light. Chemical tests may be more accurate but it is no more difficult to show them to a disadvantage than to make a disparaging statement about the biologic tests.

Which test gives more assurance, to weigh or titrate the alkaloid or to test its activity on an animal in the same sense that it is active as a medicinal agent? The opium content of a solution is measured by the amount of sulphuric acid required to neutralize while the identification of the active agent depends on the color developed when in contact with the concentrated acid.

To the uninitiated would it not be more reassuring if he were informed that a solution containing opium is identified by its sedative action on the dog and its potency measured by the amount required to put the dog to sleep in comparison with that of pure morphine or opium alkaloids?

The scope of biologic assaying is sharply defined with no function other than to measure the efficiency of remedial agents which must otherwise remain of unknown potency.

The applicability of biologic tests, however, is unlimited if taken in the qualitative sense. By no other means can one more certainly identify the active agent of a drug or determine whether a drug has an active principle.

For example, in working with a solution containing strychnine it can readily be determined by tests on animals whether a certain chemical manipulation has affected the active agent materially.

Biologic tests have still another function which is two-fold. For example, a statement was recently made that "The daily proof of the poor absorbability of strophanthus is had in the fact that the dose given in the Pharmacopoeia is the same as that for digitalis though the Pharmacopoeia requires that strophanthus shall be just one hundred times as active as digitalis."

This investigator failed to distinguish between two methods of administration which gives the complete explanation of this apparent phenomenon. Digitalis by mouth is less readily broken up in the stomach than is strophanthus, which must be administered in a relatively enormous dose in order that any may be absorbed before being acted on by the digestive ferments. When the active agents are intravenously administered, for example, into cats, dogs or pigs, comparable to that used in applying the frog assay method, or the cat method, the ratio of activities is not greatly different from the U. S. P. Standards.

This is a pharmacologic experiment which clearly demonstrates, first, the approximate correctness of the assay process; second, the proper method of using the different members of the digitalis series to get the full activity, promptly and without cumulative effects. Without animal experimentation neither of these facts could have been demonstrated.

Careful scrutiny of the ground which should be covered by the biologic assay processes and elimination of features which have no place there will do much to clear away certain logical criticisms.

Pharmacology is the study of the action of drugs by use of animals.

Biologic assaying is the use of animals as a means of standardizing drugs by comparing the action of two samples of the same drug, one of which is of known activity.

If we adhere strictly to this definition of the biologic assay it will eliminate most of the objections which have been most raised.

There is no crying demand for information based on animal experimentation as to proper human dosage. Each individual is a separate problem for the physician and no elaborately worked-out dosage on the cat or other animal is of more than academic interest. But every one who is familiar with the variability of the crude drug and with the opportunities for errors and loss in preparing extracts knows that some method of standardization must be applied if possible. And what is more logical than a biologic test which not only measures effectiveness, although sometimes crudely, but also follows closely the therapeutic action of the drug?