In this connection it may be pointed out that if both units are calculated from a minimum effective total dose that the very convenient value of 10 units per Gm. would be a reasonable minimum standard of activity for both vitamins in Cod Liver Oil. Such a figure for the minimum standard would have the further advantage in that assay results could be directly converted into per cent of U. S. P. minimum standard by simply multiplying the number of units per Gm. by the figure 10, for example, if a sample of Cod Liver Oil assayed 13.3 units of vitamin D and 15 units of vitamin A per Gm. it would have a vitamin D content of 133% and a vitamin A content of 150% U. S. P. minimum standard.

A very practical and significant question arises in connection with the quantitative interpretation of the assay data in both the vitamin A and vitamin D assays. That is—shall the deviation of response which is a biological occurrence common to any bio-assay be made use of in the evaluation of the results or shall it be ignored. This fact of deviation is made use of in the standard method for the assay of irradiated ergosterol in that the method specifies the unit dose in the terms of a definite percentage of positive responses—*i. e.*, 60%. The quantitative importance of also including such a specification in the vitamin A assay cannot be too strongly emphasized.

It is of the highest importance that the required methods for the assay of Cod Liver Oil both for vitamin A and vitamin D be provided in U. S. P. XI. This is of especial importance in view of the great variation in the labeled statement of many brands of Cod Liver Oil, due to the fact that the various firms distributing such oil have established different standards for vitamin potency. It would also seem desirable that the U. S. P. method require a uniform method of labeling, preferably in terms of the number of units per one Gm. of oil and should not permit a labeled statement as to the number of units per ounce, multiple of a gram or any fraction of the permitted figure. Such variations in the labeled statement result in confusion not only to the consumer, but also to the druggist and physician.

NEW BRUNSWICK, N. J.

THE RELAXANT ACTION OF BENZYL DERIVATIVES.*

BY LEWIS C. BRITT AND E. V. LYNN.

We are all familiar with the facts surrounding the introduction of benzyl benzoate as a relaxant of smooth muscle. On the basis of extensive experiments on animals and of some clinical data, Macht (1) in 1918 proposed the use of this compound and other benzyl derivatives as remedies in any condition of heightened tonus, such as uterine colic, vesical spasm, angiospastic conditions, spastic constipation, hiccup, etc. His work was apparently substantiated in practical therapeutics by a number of other observers (2), but the evidence therefor was obtained largely without controls and, hence, liable to considerable misinterpretation. The end-result, however, was an almost immediate adoption of the drugs by a considerable share of the medical profession. If many of the early written reports could be taken literally, benzyl benzoate and its relatives might well be ranked as panaceas for any disorder of smooth muscle due to spasm or increased tonicity.

* Scientific Section, A. PH. A., Baltimore meeting, 1930.

In spite of the first enthusiasm, there is no question but that practitioners are becoming much more doubtful of favorable effects. Prescriptions calling for the benzyl derivatives are decidedly less common and very often one can hear some physician express an opinion that they are of little if any value. Indeed, a survey of the literature shows that there are now few clincal reports of any kind, compared to the very voluminous records of a few years ago. In whatever way we approach the subject, we are led to the conclusion that these drugs do not possess great worth in the treatment of spasmodic conditions of smooth muscle.

On the other hand, various investigators have examined the experimental evidence and corroborated the results in great part (3). It is certain that the benzyl derivatives do relax unstriated muscles *in vitro* and most of the same muscles *in situ*. The intestines, uterus, blood vessels and bronchi are thus affected by local application. When the drugs are administered orally or hypodermically, however, the uniformity of findings is not as good, and the only way of correlating the various investigations is to take dosage and mode of administering into account. The effect on any muscle is directly proportional to the amount given; hence, some of the failures may have been due to inadequate dosage.

In order to throw some light on this subject, we have instituted the experiments which are briefly reported here. The anticipated possibilities in therapy have fallen far below expectations and it seemed advisable to determine the reasons. Errors in the original technic have been suggested, but it is hardly possible that benzyl derivatives do not have relaxing action on direct contact. All of them, however, are handicapped by insolubility in aqueous media, so a few experiments were made with benzyl benzoate on intestinal muscle, comparing its effects with those of two soluble compounds now available, benzyphos and benzycin.

The method and amounts of materials used were as nearly identical with those of the original experiments as could be followed (1). A segment from the intestine of a cat, rat or rabbit was removed and suspended in oxygenated Locke-Ringer solution warmed to, and maintained at, 38° C. throughout the test. The intestinal tissue was attached by a light silk thread to a writing lever and the movements of the muscle were recorded on a slowly revolving drum. When the natural contractions were well established, the drugs, or solutions of them, also warmed to 38° C., were added and the effects were determined by changes in the tracings of the kymographic record. For the sake of brevity, these tracings are not here recorded.

Critics of benzyl therapy have frequently referred to the alcoholic solvent as a possible source of relaxant effects from preparations of benzyl benzoate. Repeated trials on various muscles have shown us that alcohol does actually reduce the tonus, when directly applied. This is in contradiction to the report that "control experiments on excised tissues with ethyl alcohol showed that the action of phenylmethylol on smooth muscle is due to the benzyl grouping and not to the OH component." A 20 per cent benzyl benzoate in 70 per cent alcohol, which is a marketed product, gives practically the same reaction.

Pure benzyl benzoate produced little apparent effect on the intestinal contraction when merely dropped into the Locke-Ringer solution, because the solubility is almost nil. This is not, of course, a legitimate test of its action, since the tissue practically escaped contact with the oil.

In view of the fact that emulsions are commercial articles, such a product was used in the same manner. The effect was, in a way, comparable to that of the alcoholic solution. That acacia itself is not responsible for any of the relaxation was shown by the fact that preparations of it alone produced a primary stimulation of the muscle, which gradually returned to normal, the stimulation probably being due to natural acidity. We can conclude that benzyl benzoate had definite relaxing power on intestinal muscle.

Benzycin and benzyphos, two water-soluble preparations, were tried on similar segments, and the results demonstrated that they are even more active in reducing the muscular tonus. That this is not due to the residual phosphoric acid, in the case of benzyphos, was shown by a trial with sodium phosphate which gave a primary stimulation, followed by an approximate return to normal after a second or two. Dibenzyl, which is insoluble in water, gave no effect on the segment of intestinal muscle.

Assuming, therefore, that the benzyl compounds are actually relaxant to the muscular tissue, and that they are not effective clinically, the explanation must lie in non-absorption or in insufficient dosage. In order to test the latter point, some experiments were performed using varying amounts of benzycin. To each of equal portions of Locke-Ringer solution containing the normal muscle, varied amounts of 1 per cent solution of the drug were added. After several trials, it was found that the minimum quantity is represented by something between 10 cc. and 15 cc. of this solution. Selecting 10 cc. of 1 per cent benzycin as being a minimum necessary to produce any muscular relaxation, we calculate that the final mixture of 35 cc. contained approximately 0.1 Gm. or about 0.3 per cent of the total solution. Since the dosage of benzycin which is recommended is only 0.3 to 1.0 Gm., it may easily be imagined that at any point in the body we would not have more than a small fraction of this essential 0.3 Gm. per 100 cc. of body fluid, unless of course the drug collected at this given point. It is, indeed, reasonable to conclude from these results that the dosage might need to be at least an ounce or more. The limitations of the experiments are apparent, but the real explanation of non-effectiveness after oral or hypodermic administration undoubtedly lies in insufficient dosage.

There still remains the question of how much is absorbed after oral administration. Snapper, Grünbaum and Starkop have already reported (4) work along this line, their results showing that 60 to 90 per cent of the benzyl derivatives given can be recovered in the urine as hippuric acid. Our own experiments were carried out on a few human subjects by giving to them definite quantities of the benzyl compounds and noting the changes in amounts of hippuric acid in the urine. Previously the normal daily excretion of the acid was determined over two days during a constant diet. The results of these tests were anomalous and not at all uniform, due in part to unavoidable variations in food intake. Typical results of two determinations are given. (We hope to continue this phase in the near future.)

	Hippuric acid.	Per Cent of Increase. Found.	Calculated.
Normal, first day	0.94		
Second day	0.94		
Benzyphos, 1 Gm.	1.13	20.2	127.6
Benzyl benzoate, 1 Gm.	3.33	254.2	179.7

Incidentally it was found that benzycin appears to be strongly diuretic; in all of the subjects the quantity of urine was more than doubled. With benzyl benzoate the increase was only 40 per cent. The fairly large doses taken in all cases were found to have no apparent ill-effects.

In conclusion, it might be noted that the benzyl esters are probably hydrolyzed before they have proceeded very far after ingestion. The part of the canal in which absorption takes place will undoubtedly influence the physiological action very profoundly. If hydroylsis occurs in the intestinal tract, one might expect muscular relaxation only at some point above this and certainly not after absorption of the products, unless the latter have such an action. Otherwise, a simple mixture of benzoates, phosphates or succinates with benzyl alcohol would be as efficient.

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THE APPLICATION OF STATISTICAL METHODS TO PHARMACEUTICAL RESEARCH. I. MEASURES OF ACCURACY.*

BY JAMES C. MUNCH.

The key-note of quantitative investigations is accuracy. Many investigations have been incomplete, and faulty conclusions have been drawn from an inadequate number of results, because of the neglect of this important factor. The variability of animals has been used as an excuse for divergent results from time immemorial. On the other hand, the accidental coincidence of two successive tests has been accepted as conclusive proof of correctness of results, irrespective of the nature or number of variables involved.

Simple mathematical procedures have been developed for measuring the accuracy of results. This type of mathematics has been employed so extensively in connection with the investigations of biometricians, physicists and actuaries that the nomenclature of this field follows their terminology (1, 2, 3, 4, 5, 6, 7, 8, 9, 13). This may explain the reluctance of workers in other fields to apply this type of mathematics to the interpretation of research results (11).

Two types of variations must be differentiated: (*1*) constant errors and (*2*) variable errors. Constant errors result from defects in apparatus, incorrect graduation of equipment, erroneous calibration of weights, etc. A constant error will be produced in all measurements with faulty equipment. Increasing the number of observations will have no effect in correction of a constant error. The presence

* Scientific Section, A. PH. A., Baltimore meeting, 1930.