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FURTHER STUDIES OF THE EFFECT
OF SAPONIN ON INTESTINAL ABSORPTION

A THESIS
SUBMITTED IN PARTIAL FULFILMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

DEPARTMENT OF PHYSIOLOGY

BY
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VITA

- 1907 - Born, June 6, in the town of Montegallo, province of Ascoli-Piceno, Italy.
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INTRODUCTION

In recent years, much work has been done, chiefly in the laboratories at Innsbruck, tending to show that saponin aids in the intestinal absorption of various substances. It has been found that the toxic oral dose of various drugs was greatly reduced when administered simultaneously with saponin, whereas the parenteral toxic dose was unaffected. Some of the most striking results were obtained by the following investigators.

In 1925, Kofler and Kaurek (1) found that they could produce systolic arrest of the frog heart with one-third of the ordinary oral dose of strophanthin and one-fifth of the ordinary oral dose of digitoxin when these substances were administered simultaneously with saponin. The effective parenteral dose remained unchanged, being 0.001 mg. The oral dose was reduced from 0.09 mg. without saponin to 0.0027 mg. with saponin. Similar results were obtained with mice, but were not as striking, while the effect on rabbits was doubtful.

Lasch (2) in 1926 made a series of studies on the effect of saponin on the absorption of calcium chloride from surviving loops of intestine. He suspended loops of the small intestine filled with a solution of calcium chloride, containing a known amount of calcium, in oxygenated Ringer's solution. Samples of the intestinal content were withdrawn at various

intervals and the percentage of calcium determined. The procedure was then repeated but with the addition of saponin to the calcium chloride solution. He found that without the aid of saponin, the amount of calcium absorbed ranged from 5.8 per cent in fifteen minutes to 18.2 per cent in one hundred minutes, whereas if saponin was added, the amount of calcium absorbed ranged from 13.0 per cent in fifteen minutes to 36.5 per cent in one hundred minutes.

Lasch and Brugel (3) in 1926 investigated the effect of saponin on the intestinal absorption of sugar solutions. Here also isolated segments of the small intestine of the guinea pig were used. The segments were filled with a sugar solution of known concentration. At various intervals of time samples were withdrawn and the sugar content determined. They found that without saponin the amount of sugar absorbed ranged from 0.75 per cent in fifteen minutes to 18.0 per cent, the maximum, in one hundred and thirty-five minutes. With the addition of saponin, the absorption ranged from 15.0 per cent in fifteen minutes to 53.0 per cent, the maximum, in one hundred minutes.

Again in 1927, Lasch and Brugel (4) studied the effect of saponin on absorption using insulin as an index of absorption. It was found that insulin would produce the same effects on blood sugar when administered orally with saponin as when it was injected subcutaneously alone. These results were obtained on rabbits, dogs and man. The authors concluded that

saponin, besides aiding in the absorption, also serves to protect the insulin from the action of pepsin and trypsin.

Flasch (5), also in 1927, performed the same type of experiments and he too, found that insulin administered orally with saponin would produce a typical effect, but only after a twelve hour fast.

Dingemans and Laqueur (6) repeated the experiments of Lasch and Brugel (4) but could not confirm their results.

Annau and Herglotz (7) in 1927 using saponins from several sources found that they could administer small quantities of curare, picrotoxin, or strychnine to the frog without producing any toxic symptoms. However, when the same doses were administered with saponin symptoms of severe intoxication would be produced. Similar results were obtained when morphine, cocaine and magnesium chloride were administered to rabbits, with and without saponin.

Kofler and Fischer (8) in 1928 were able to produce narcosis in mice and frogs with small quantities of magnesium sulphate, when saponin was administered simultaneously, or if the saponin was administered a short time before the magnesium sulphate. The narcosis could not be produced if the magnesium was administered later than three hours after the saponin.

The work of Endo, Mizoguchi and Naito (9) showed that saponin increased the absorption of potassium iodide, reduced iron, magnesium sulphate, cholera and typhoid bacilli and cholera and typhoid immune sera.

Other investigations yielding similar results are those by Lasch and Brugel (10) showing greater absorption of dextrose with than without saponin; by Kofler and Fischer (11) showing an increased absorption of curare with saponin; and by Petschacher (12) on resorption of pituitary extract with saponin.

A little more pertinent to the problem at hand is the work of Berger, Tropper and Rischer (13) who investigated the effect of saponin on the intestinal absorption of calcium salts in man. Five grams of calcium lactate were administered in the morning to the fasting subject and blood drawn at two hour intervals for analysis of blood calcium. Several days later the experiment was repeated with the addition of 0.1 gram of saponin (Merck). Without saponin there occurred no appreciable rise in the blood calcium, but when saponin was also given they found that the blood calcium rose from 7 to 41 per cent of the initial value. The specific values obtained in per cent of the initial blood calcium values were 7, 10, 12, 15, 22, 23, 27, and 41 per cent.

The results of the aforementioned investigators tend to show, very strikingly, that saponin is of considerable aid to absorption. With this in mind it was decided to study the effect of administering orally, small amounts of saponin and calcium to parathyroidectomized dogs. However, since no data was available on the effect of saponin on absorption in normal

dogs, it was thought best to make some investigations on normal animals. The results of the first series of studies made by Sternasty in 1930 were somewhat of a surprise. Consequently, a reinvestigation was made and it is this second investigation that is herein discussed.

The saponin used in this work was obtained from Merck and from Clarence Morgan. That obtained from Merck is extracted from soapwort and is of the quality labeled purum albissimum. In general, the saponins are of numerous varieties and are widely distributed in over four hundred plants belonging to about fifty different orders. They belong to a class of substances known as the glucosides, so-called because on acid hydrolysis they yield a sugar, in most cases glucose, and a physiologically active substance. In the case of saponin, these latter substances are called sapogenins and often are of a polyhydroxylactone nature.

The characteristic properties of the saponins are: They are white or cream coloured powders which in most cases are colloidal in nature. In water they form a clear solution which froths on agitation. They are insoluble in ether, chloroform, benzene, and cold ethyl alcohol, but freely soluble in hot alcohol. They have a very bitter and acrid taste. In powder form they are very irritating to the mucous membranes, especially of the nose. They are toxic, particularly to cold blooded animals, and are strong poisons to fish. They are also well

known for their hemolytic effect on erythrocytes.

The saponins are obtained from the roots, leaves and seeds of plants by extraction with alcohol or water. If water is used, special precautions must be taken to destroy the accompanying enzyme which will cause hydrolysis. The extract is precipitated with neutral or basic lead acetate. The precipitate is decomposed and the solution evaporated. The residue is then extracted with chloroform and precipitated with ether.

PROCEDURE

The dogs used in these experiments were of medium size, to facilitate handling, and were all in very good physical condition. The day before the experiment, the animal was placed in a cage and starved for twenty to thirty hours so that the stomach and small intestine would be free of material and thereby reduce the possibility of any interference with the absorption of the calcium lactate and also to allow for the fullest possible action of the saponin on the intestinal mucous membrane. On the morning of the experiment a ten c.c. sample of normal blood was drawn from the saphenous vein. Immediately after drawing the normal sample a 5 per cent solution of calcium lactate (Mallinckrodt) was administered, the dose being 20 c.c. per kilogram body weight. In the last seven experiments a 2.5 per cent solution of calcium lactate was used. The difference in results obtained will be discussed later. One hour after the administration of the calcium the second sample of

blood was drawn, and all subsequent samples at two hour intervals. In all, five samples of blood were drawn, the experiment running over a period of seven hours. One week later, the same animal was used again and put through the same procedure, but in addition saponin was administered simultaneously with the calcium lactate in such a quantity as to make a 0.01 per cent solution. After the first five experiments the dose of saponin was changed so that the concentration was 0.05 per cent.

In all cases the blood was drawn from the saphenous vein. In the second part of the last experiment, because of the condition of the vein, it was drawn directly from the heart. The blood was obtained by puncture of the vein with a hypodermic needle on a 10 c.c. syringe, which contained approximately 3 mg. of heparin (Hynson, Westcott & Dunning) to prevent clotting. After each drawing the syringe was thoroughly cleaned with soap and water, rinsed three times, once with tap and twice with distilled water, and finally dried in an electric oven.

Blood calcium was analyzed by the method of Kramer and Tisdall as modified by Tweedy and Koch (14). This was also slightly modified so as to follow the suggestion of Pincussen and Schimmelpfeng that twenty-four hours be allowed for the precipitation of the calcium. In general, the modified Kramer-Tisdall method is as follows: The drawn blood is centrifuged at high speed for fifteen minutes so as to rid the plasma of

all formed elements. A 2 c.c. sample of the plasma, measured with a standard 2 c.c. pipette, is transferred to another centrifuge tube and treated with 1c.c. of a 4 per cent solution of ammonium oxalate, which precipitates the calcium as calcium oxalate. The tube is then set aside for twenty-four hours to allow for the precipitation of all the ionizable calcium. At the end of that time the contents are centrifuged again, this time for ten minutes. The supernatant fluid is drawn off leaving the precipitated calcium oxalate at the bottom of the tube. The precipitate is washed three times with a 0.5 per cent solution ammonium hydroxide saturated with calcium oxalate. After the third washing, the precipitate is dissolved in 2 c.c. of N sulphuric acid, heated, and titrated while warm with N/100 potassium permanganate.

TABULATED RESULTS

Table I

Calcium lactate, 5.0 per cent solution, 20 c.c. per kilo body weight.

Exp. No.	Controls		Saponin added, 0.01 %	
	Initial Blood Ca	Maximum Blood Ca	Initial Blood Ca	Maximum Blood Ca
1	12.966	13.522	11.566	14.032
2	11.634	13.594	11.099	13.570
3	12.720	15.053	13.235	16.060
4	11.482	14.130	11.370	13.997
5	11.370	13.874	12.101	14.906

Table II

Calcium lactate, 2.5 per cent solution, 20 c.c. per kilo body weight.

Exp. No.	Controls		Saponin added, 0.05 %	
	Initial Blood Ca	Maximum Blood Ca	Initial Blood Ca	Maximum Blood Ca
6	11.807	12.097	10.820	12.902
7	11.124	11.910	11.443	12.327
8	11.954	13.113	12.524	13.211
9	12.990	14.341	13.383	13.835
10	12.337	13.457	12.033	14.243
11	12.938	15.225	11.962	15.092
12	11.512	13.477	10.510	12.917

DISCUSSION

The above results, with those of Sternastj's experiments, indicate that the presence of saponin has little or no influence on the rate of absorption of calcium from the intestine of the dog. This is contrary to the positive findings of Lasch (2) on the isolated intestine of the guinea pig; of Berger, Tropper and Rischer (13) on human subjects; and of Kofler and Fischer (8) in mice. A species difference of susceptibility might be assumed to exist so that absorption may be affected more favorably in some species than in others. Apparently it aids absorption in frogs, mice, and human subjects, and in the

isolated intestine of the guinea pig, while it does not seem to have any effect in the dog. An example of the difference of effect in different species are the findings of Kofler in regard to the symptoms produced in man and the dog. It was found that in the dog saponin will produce diarrhea due to a marked intestinal irritation, whereas in man it does not have this effect. Wokes, however, has repeated and failed to confirm the work of Kofler and Fischer (8) on mice. And, in view of the marked variability of the blood calcium values found by Berger, Tropper and Rischer, it would hardly seem safe to draw definite conclusions from such a small number of experiments. Our experiments were more numerous and the blood calcium curves more uniform and we therefore feel that our results are a little more reliable. Like Wokes (15), we used two preparations of saponin, Merck's (which is the same as that used by Kofler and Fischer) and another of British make, Clarence Morgan's. Another statement which is in support of our results was made by Lasch (2) who stated that the effect of saponin on absorption in puppies was doubtful. Also, Petschacher and Nageeb (16) were unable to demonstrate satisfactorily in man, any hyperglycemia due to glucose per os after saponin, although they were able to show that saponin increases the absorption of various drugs from the alimentary canal.

In experiments one and five of table I and six and ten of table II there is seen a rise of 1 mg. or slightly more of calcium in favor of the saponin experiments, but in just as

many cases there was a slight difference in favor of the control experiments. In no cases however, were any such differences observed as were obtained by other investigators. Our results, subjected to statistical treatment gave us the following values: The mean normal calcium value was 11.4 mg with a probable error of 0.069 mg. The mean maximal value value of the blood calcium following administration of calcium lactate alone was 13.65 mg. with a probable error of 0.157 mg. The mean maximal value for the experiments in which saponin was also administered was 13.92 mg. of calcium with a probable error of 0.134 mg. The difference is so slight that it is insignificant, especially in view of the well-known fact that the blood calcium content varies in the same animal at various intervals.

The blood calcium curve in all of the experiments was practically the same. However, a slight difference might be pointed out. In all the experiments in which a 5 per cent solution of calcium lactate was used, the peak in blood calcium was reached in three hours, whereas in the experiments in which a 2.5 per cent solution was used with a higher concentration of saponin, the peak was reached in one hour in three of the experiments.

A possible explanation for the failure of the saponin to increase absorption may lie in the work of Kofler and Fischer (17). They made a comparison of the power of a number of different saponins to increase the absorption of curare from the

alimentary tract of the frog. They compared digitoxin, sapotoxin and saponin from horse-chestnuts, gualiacum and solanin. All except the saponin from the gualiacum increased the rate of absorption. They also found that when the saponins were combined with cholesterol their power of increasing absorption was much reduced or entirely abolished.

In regard to the dose of calcium administered, the objection might be raised that such a large amount of calcium lactate might have caused a saturation of the blood so that the effect of the saponin was not apparent when it was added to the calcium lactate solution. Such is not the case as can readily be seen by glancing at table II. In the experiments tabulated there, a 2.5 per cent solution of calcium lactate was used, which is half the concentration of that used in the experiments recorded in table I. The results show that a smaller amount of calcium is absorbed in the controls and that no greater absorption occurs in those experiments in which saponin was also administered. The calcium absorption curves in both tables are almost identical. Also, it might be stated that in animals fed with parathyroid extract, the blood calcium values are much higher than any of those obtained by us even with the 5.0 per cent solution of calcium lactate.

SUMMARY

Calcium lactate, in 5.0 per cent and 2.5 per cent solutions were administered to dogs so that they received 1.0 gm. and 0.5 gm. per kilo body weight. This was administered to the dogs twice, once alone and a week later with saponin. The saponin was administered in doses of 2 mg. and 10 mg per kilo body weight. The saponin used was Merck's purified and Clarence Morgan's. The results show that saponin, under these conditions, does not increase absorption as determined by increases in the plasma calcium content.

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