ON THE CHEMISTRY AND BIO-CHEMICAL AND PHYSIOLOGICAL PROPERTIES OF A SAPO-GLUCOSIDE OBTAINED FROM THE SEEDS OF *BASSIA LONGIFOLIA* (MOWRAH SEEDS).¹

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INTRODUCTION

The seeds of *Bassia longifolia* are imported into Liverpool from India because of a valuable oil which they contain in large quantity. The oil is a clear yellow semi-solid fat or butter at ordinary temperature and is excellent for the manufacture of the better classes of soaps. It is expressed by pressure of the crushed seed while hot, and the residue, known as Mowrah meal, is the material which we have used for our work, as it contains all of the sapo-glucoside² in which we are interested on account of certain physiological actions closely resembling those of the cardiac tonics of the digitalis group of drugs. The meal, moreover, being almost fat-free, and already crushed, saves a good deal of preliminary labour which must be gone through if the whole untreated seeds are used as the experimental material.

The tree from which the seed is obtained is found in Central India, its habitat extending roughly in a belt about 120 miles broad from Bombay to Calcutta.

It is stated by Watts, in his 'Dictionary of Economic Products of India,' that the tree is of great importance to the natives and that any condition interfering with the Mowrah crop may cause a famine in the district. The flower is of a cream colour and appears in March and April,

1. The expenses of this research have been in part defrayed by a grant from the Government Grant Committee of the Royal Society.

2. We have introduced the word sapo-glucoside to designate the large number of substances belonging to the glucoside class which also show the properties of 'saponin,' and act physically like soaps. It is confusing that the name of one substance, saponin, should be applied to dozens of different compounds of different constitution. The term sapo-glucoside conveniently shows that these bodies belong to the class of the glucosides, and possess soap-like properties. To these soap-like properties attach other important powers such as haemolytics and cardiac drugs, which they share with soaps and bile salts.

before the leaves. The orange-coloured fruit, which has somewhat the appearance of an apricot and is from one to two inches long, ripens about three months after the flowers have fallen. Each fruit contains from one to four of the Mowrah seeds. These seeds yield the meal with which we have worked; they resemble in colour, size, and appearance small almond seeds.

Our attention was drawn to this product for two reasons, both connected with the presence of the sapo-glucoside. One reason was of commercial; the other of medical interest.

The usual commercial use made of the spent seeds used for obtaining oils for different purposes is to convert them into a basis for feeding-cakes for cattle—a purpose which they serve most admirably, as they contain a good deal of nutrient matter in an easily digestible form, and the process of crushing only enhances this nutritive value.

In the case of Mowrah seeds, however, the high market value of the oil is somewhat discounted by the difficulty of using the meal in appreciable amount for cattle-cake manufacture. For the sapo-glucoside in which we are mainly interested possesses such a bitter taste that cattle will not eat cake containing any high percentage of the meal. The extreme bitterness of the sapo-glucoside is shown by the fact that this is so, although the meal only contains approximately one per cent. of the sapo-glucoside.

The other irritant and poisonous properties of the sapo-glucoside which we are about to record have also made manufacturers chary of using the meal in cake manufacture on account of possible litigation.

As we shall see later, however, the sapo-glucoside when taken by the mouth is much less poisonous than when it is administered subcutaneously or by intravenous injection. Cattle or laboratory animals compelled to eat it in considerable amount do not show any obvious signs of poisoning, and in small amounts in cake it might possibly be more advantageous than deleterious.

The other reason which brought the Mowrah meal before us, was the effect of the fine dust from it, after it had been crushed and the fat extracted, upon the mucous membranes or any abrasions of the skin of workmen employed in manipulating it, especially in any way which involved stirring it up, such as shovelling.

On cut surfaces, or mucous surfaces, it acts usually as a most powerful irritant, preventing cuts from healing by producing inflammation, sloughing and erosion; and on the mucous surfaces it causes sneezing and

coughing in those not inured to it, with a hot irritating sensation in the throat, followed by catarrh and inflammation.

Our investigations soon demonstrated that both the bitterness of the meal and its irritant action were due to the same substance present in small amount in it. This body was then found to be a glucoside, and shown by its frothing in aqueous solution and powerful haemolytic action on red blood corpuscles to be a sapo-glucoside. Both the glucoside itself and its organic acid obtained on hydrolysis fortunately proved to have such solubilities in different solvents, as enabled us readily to separate them in a state of purity, and follow up both chemical constitution and physiological properties of the pure sapo-glucoside.

The interesting discovery was also made that the action on the cardiac mechanism of the sapo-glucoside very closely resembles that of the digitalis group of cardiac tonics, the heart being slowed and the strength of its beat increased by small doses.

According to Waller,¹ activity in digitalis preparations is associated with the presence of the physical property of frothing in the solutions; and, also, the action of saponin on a nerve-muscle preparation is similar to that of digitalis.

In our opinion, this large group of allied substances, hitherto called 'saponins,' which we have termed sapo-glucosides, owe their cardiac action to a physico-chemical action on the cardiac lecithides, similar or identical in kind to that which causes them all to produce laking of the red corpuscles by their soap-like action on the lecithide of the corpuscular stroma. The action is probably due to a molecular union, or adsorption, preceding a solvent action.

As far as we are aware, the particular sapo-glucoside with which we have here experimented has not been previously described or investigated, and we have ventured to name it Mowrin from its origin in Mowrah seeds, while the organic acid resulting from its hydrolysis we have called Mowric acid.

The work can be best described in the following sections, and, since we have not all been engaged in the work of each section, the names of the authors responsible are given under each sectional heading:—

- A. Organic and physical chemistry.
- B. General physiological properties.
- C. Haemolytic action.
- D. Action on the isolated mammalian heart.

1. *Proc. Physiol. Soc.*, Dec., 1908; *Journ. of Physiol.*, Vol. XXXVIII.

SECTION A. ORGANIC AND PHYSICAL CHEMISTRY

BY BENJAMIN MOORE AND T. A. WEBSTER

Preliminary investigation. Samples of the meal were extracted with hot water and hot alcohol respectively. The hot alcoholic extract was evaporated to dryness and the residue dissolved in water. Portions of aqueous and alcoholic extracts injected into animals both killed rapidly, showing that the toxic substance was soluble in both water and alcohol.

The material from both extracts was found to reduce Fehling's solution, and showed other reactions of a reducing sugar. On hydrolysis, by boiling with hydrochloric acid, a white gelatinous precipitate came down, rapidly setting into a jelly in even moderately dilute solutions. The filtrate from this precipitate was found to contain a still larger amount of sugar, and from this it was suspected that the active body was a glucoside.

The clear filtered alcoholic extract from the meal was treated with ether (1 vol. of alcohol to 2 vols. ether), which gave rise to a precipitate, as a white voluminous mass. This precipitate was filtered off rapidly and dried *in vacuo* over sulphuric acid. The product thus obtained was a *pale* yellow amorphous powder, soluble in water in all proportions and sparingly soluble in absolute alcohol.

Tested physiologically this substance was found to be intensely active. It possessed an exceedingly bitter taste, frothed strongly in aqueous solution, and laked blood corpuscles almost instantaneously. Injected into frogs it caused slowing of the heart, with rapid death.

A solution in water of this powder was found to give all the reactions of a glucoside. On hydrolysis by acid, it gave a white gelatinous precipitate, turning the whole fluid into a thick solid jelly, just as did the original extracts of the meal; and the filtrate showed strong reducing properties and gave a typical glucosazone. In addition to the insoluble acid and the reducing sugar, a volatile substance with a distinct smell resembling that of fusel oil was formed; the smell of this substance is most typical and is always observed when the glucoside is hydrolysed.

The sapo-glucoside, as precipitated by ether and taken up in water, gives a beautiful colour reaction, which appears to occur as a class reaction with all the sapo-glucosides. This colour is a fine purple, exactly like that given by Pettenkofer's test for bile salts, and is obtained similarly by the addition, either to particles of the powder or aqueous solutions of the same, of strong sulphuric acid.

With aqueous solutions, the test is best carried out in the contact-ring form in a test-tube, a beautiful purple zone developing at the junctional layer between the strong sulphuric acid and the aqueous solution. The same result is got by adding a few particles of the solid powder to strong sulphuric acid, when the acid turns first red and then a deep purple throughout. On dilution, the colour is precipitated out, as a dirty greyish precipitate, and it is insoluble in all the usual solvents.

The similarity of this test to the Pettenkofer test is remarkable, and, as we shall see later, furfurol, which is necessary in the Pettenkofer test, forms a part of the sapo-glucoside molecule, or at least is set free in quantity on hydrolysis of the mowrin.

We may also pause here to point out how similar the above separation is to the precipitation of the bile salts in the Plattner method for obtaining the bile salts. Both this sapo-glucoside and the bile salts are soluble in alcohol and in water, and insoluble in ether.

The above colour test is probably due to some common molecular grouping in both bile salts and the sapo-glucoside. Both the cholalic acid of the bile, and the acid of this and other sapo-glucosides, are complicated organic acids, as we shall see, unsaturated and hydroxylated, in that they can additively take up halogen or acetyl groups; all of them reduce the surface tension of water, as shown by the frothing they produce in aqueous solutions; all rapidly haemolyse red blood corpuscles; and all slow the beat of the heart.

The precipitate produced in the Mowrin solutions, on hydrolysis by acids, was easily soluble in alcohol. It was shown to be an organic acid by dissolving readily in water on treatment with alkali and reprecipitation on adding excess of acid again. It was also shown to have a low but quite definite neutralization figure.

This preliminary investigation having pointed out the method of attack, the preparation and purification of the mowrin was carried out as follows:—

Method of isolation. Two to three kilograms of the meal were taken and placed in three large beakers, the contents of which were treated successively with several quantities of cold absolute alcohol. Filtration was at first effected through calico, and the filtrate from beaker No. 1 was used to extract the meal in No. 2, and so on, to No. 3. Fresh alcohol being always added first to No. 1, in successive quantities, until No. 1 was about exhausted, as shown by absence of ether precipitation in a test of the alcoholic filtrate, when the meal in No. 1 was discarded, and so on.

The alcoholic extracts were then combined, and evaporated down to about one-third of their original bulk. The clear extract on cooling was treated with double its volume of ether, and the precipitated glucoside was then filtered off, by means of a Buchner funnel, well washed with ether, and set at once to dry *in vacuo* over sulphuric acid. It was found that if the alcohol extract was too concentrated, the glucoside, instead of coming down as a white flocculent precipitate, was thrown out as a brownish gummy mass, which adhered to the sides of the vessel with great tenacity. This appears to be due to the great attraction of the glucoside for water, in which it is soluble in all proportions, and takes up greedily from the air. Accordingly, when there is not a sufficient excess of absolute alcohol to hold firmly the small amount of water present, this comes out with the precipitated sapo-glucoside. So long as there is excess of alcohol, the glucoside is precipitated as a white flocculent mass. In its precipitation in this latter form the sapo-glucoside appears to hold in molecular union, or in some physical fashion, a large amount of alcohol or ether, for although the precipitated mass is very bulky it dries up to a very small amount. In fact, the precipitate rapidly becomes semi-fluid almost before it can be got into the desiccator, and *in vacuo* begins to boil, leaving behind a yellowish white body. This is then purified by repeating the process of solution in alcohol and precipitation with ether, until finally an almost pure white substance is obtained.

This substance is entirely organic, leaving no ash on incinerating on platinum foil. Qualitative tests, and a Kjeldahl analysis giving negative results, demonstrated that it contained no nitrogen, and by exclusive qualitative testing, it was shown to consist of only carbon, hydrogen and oxygen. After drying to a constant weight *in vacuo* over sulphuric acid, the substance was combusted with the following results:—

Combustion No. 1,	Carbon = 51.90 %;	Hydrogen = 6.85 %;	Oxygen = 41.25 %
„ No. 2, „	50.29	„ 6.67	„ 43.04
„ No. 3, „	50.34	„ 7.32	„ 42.34
Average	... 50.84	6.95	42.21

The nearest empirical formula yielded by these figures is $C_{51}H_{84}O_{32}$; but analyses from different samples vary somewhat, and results from freezing point determinations, from bromination and from acetylation, yield quite different values. These variations we believe to be due to changes in amount of polymerization of the mowric acid, which appears to show great facility for condensing. This discussion will be resumed at the end of the description.

Properties of Mowrin. The sapo-glucoside, mowrin, is an amorphous white powder with a pale yellow tinge, soluble in all proportions in water and fairly soluble in methylic and ethylic alcohols, pyridine, aniline, and glacial acetic acid. The very strong solutions in water have the gum-like appearance of colloidal solutions, and the substance never separates as a discrete solid phase in equilibrium with clear solution, no matter how much it is concentrated. The aqueous solutions even at extreme dilutions give permanent frothes with the greatest ease.

The acid is set free on hydrolysis as a kind of hydrogel, and even a fraction of 1 per cent. concentration gives a completely solid jelly in the acid solution. For complete hydrolysis, a certain concentration of acid is required, for hydrolysis was found to be incomplete even after twelve hours with 1 per cent. hydrochloric acid. Boiling with acetic acid even up to full concentration did not give the characteristic precipitate of the mowric acid, and it was at first erroneously thought that acetic acid was not strong enough to induce the hydrolysis. It was found later, however, that the mowric acid is soluble with readiness in acetic acid, and then it was seen that the acetic acid had hydrolysed the mowrin but had held the mowric acid in solution, for the precipitate appears on diluting the solution boiled with acetic acid with a large excess of water.

Products of hydrolysis. Two grammes of mowrin were dissolved in 100 c.c. of distilled water, and hydrolysed by boiling with 50 c.c. of concentrated hydrochloric acid. In about two minutes the whole mass set solid from the separation of gelatinous mowric acid. This was diluted with distilled water and filtered, and the precipitate was thoroughly washed and then dried, *in vacuo*, over sulphuric acid.

This precipitate when first formed was of a pale pinkish white colour, but the colour rapidly darkened, and it was found that even with most careful manipulation and avoiding exposure to air it was impossible to preserve it in a white form. In the process of drying it shrunk to a mere fraction of its original bulk, and a quantity which had filled in gelatinous form a 500 c.c. beaker, when dried weighed less than one gramme. This bulky gelatinous character made work with it exceedingly difficult, and washing and filtering most tedious.

It was readily soluble in alkalies, and on acidifying was again thrown out in the same gelatinous form.

Solubilities of mowric acid. It is insoluble in water, ether, and cold hydrochloric acid, slightly soluble in hot acid and easily soluble in alcohol and glacial acetic acid. We were unable to obtain the acid in a crystalline

form. It was prepared in as pure a condition as possible, by precipitating the crude acid as above described, redissolving in alkali, reprecipitating with hydrochloric acid and washing thoroughly and drying. This product was then combusted with the following result:—

Amount of substance taken	=	0.1413 gram.
Yield of carbon-dioxide	=	0.3122 „
„ water	=	0.1121 „

This leads to percentages of C=58.57, H=8.81, O=32.62. These figures approximate closely to the empirical formula $C_5H_2O_2$ (=mol. wt. of 101); but as the saponification figure obtained by titration of the acid with deci-normal alkali in several different experiments lead on the supposition of a mono-basic acid to a molecular weight value of about 570 to 600, it is clear that this figure must be multiplied by at least six.¹

The acid is unsaturated, as shown by its changes on standing in the air, and it also is readily brominated and acetylated.

It is best brominated in solution in glacial acetic acid, and the brominated mowric acid is precipitated as a pale brown amorphous mass on diluting with water, and partially in minute crystalline form by evaporating the glacial acetic solution.

Estimated by Carius method, the bromine in the brominated product only amounts to 12.3 per cent. bromine. For an acid containing two atoms of bromine, which is about the lowest possible, this percentage of bromine leads to a molecular weight of 1,300, and indicates, therefore, a molecular weight of 1,140 for the mowric acid.

A titration of the bromo-mowric acid with deci-normal alkali gave, however, the result that 0.5 gramme only required 2 c.c. of alkali, leading to a molecular weight as mono-basic acid of 2,500, and indicating, therefore, with the above bromine percentage, a tetra-bromo-mowric acid.

An acetyl derivative prepared by boiling with acetic anhydride and precipitating with water gave, on saponification with alcoholic potash, neutralization of 30 c.c. of N/10 alkali by 0.5 gramme, which works out to 0.142 gramme of acetic acid to 0.358 of mowric acid, or 20.2 per cent. of acetyl in the acetylated acid.

This figure would give roughly three acetyl groups to each single molecule (at 600 mol. wt.) of mowric acid, or if there is a condensation of four of these, as indicated by the above bromination figures, then the more complex molecule of mowric acid would be able to take up twelve

1. In one experiment 0.154 gramme required for saponification 2.7 c.c. of N/10 alkali, leading on the supposition of a mono-basic acid to a molecular weight of 571; another experiment in which 0.2402 gramme were dissolved and titrated in alcoholic solution required 3.9 c.c. N/10 alkali, giving a molecular weight of 616.

acetyl groups or four bromine atoms; so pointing to two double linkages and to twelve hydroxyl groups in the molecule.

When these very variable indications are taken in conjunction with the molecular weights given by the cryoscopic method as seen below, it is obvious that the molecular weight must be left an open question.

The most obvious explanation which would correlate them all is that the mowrin molecule has a relatively simple constitution, but that the mowric acid on being set free polymerized very rapidly, as its physical character as a jelly also indicates.

The mowric acid gives the violet coloration with strong sulphuric acid, described above for the sapo-glucoside, and, as will be pointed out in a later section, possesses similar toxic properties.

It is a very stable body, not being perceptibly broken down into simpler bodies even by six hours' boiling with 20 per cent. sulphuric acid.

The filtrate after the hydrolysis was next examined qualitatively and quantitatively for the detection and determination of the remaining constituents of the mowrin.

The reducing sugar was found to be dextrose. It gave a typical crystalline glucosazone, melting at the proper melting point, and was distinguished from fructose by not reducing ammonium molybdate in acetic acid solution.

The third product of the hydrolysis, viz., furfural, was first recognised by the pink colour given by the vapour with aniline acetate paper. A further test was the obtaining of the phloroglucide on distilling with hydrochloric acid and treating the distillate with phloroglucinol, when the typical dark coloured phloroglucide was obtained.

As furfural can readily be obtained, under such circumstances, as a decomposition product of certain sugars, such as the pentoses, it remained doubtful whether the furfural obtained by the hydrolysis was so to be accounted for or whether it was a true and direct cleavage product of the mowrin itself. Thorough fermentation with yeast of some hydrolysed mowrin was found to leave no reducing sugar behind, as it would have done had pentoses been present, for these are not fermentable by yeast. Hence, if the furfural be due to hydrolysis of pentoses from the mowrin molecule, the breakdown must occur in the preliminary stage of all, when the mowrin molecule is first broken by the acid. For this fermentation experiment shows that there is no pentose present immediately after hydrolysis. So we may conclude that the furfural is not formed in the subsequent process of distillation with acid.

QUANTITATIVE ESTIMATION OF THE HYDROLYTIC PRODUCTS

1. *Estimation of mowric acid.* One gramme of mowrin was dissolved in 50 c.c. of distilled water and boiled for one hour with 50 c.c. of strong hydrochloric acid. The precipitated acid was filtered off, well washed with hot water, dried at 105° C. to a constant weight and weighed. Various analyses with different samples of mowrin gave results lying between 35 and 40 per cent. of mowric acid.

2. *Estimation of sugar.* The dextrose was estimated by hydrolysing a weighed quantity of mowrin with weaker hydrochloric acid, filtering off the mowric acid, boiling the neutralized filtrate with excess of Fehling's solution, filtering the cuprous oxide off on to a Gooch crucible, oxidizing and weighing, as cupric oxide. The results in various samples varied between 25 and 41 per cent. of dextrose in the mowrin.

3. *Estimation of furfural.* The furfural was estimated by the method of Kröber and Rimbach.¹ A weighed quantity of mowrin was distilled from 10 per cent. hydrochloric acid until the distillate amounted to 200 c.c., distilling at the rate of five or six drops a minute.

One gramme of phloroglucinol dissolved in 200 c.c. of 10 per cent. hydrochloric acid was then added to the distillate and left standing over night. The precipitated phloroglucide was then filtered on to a Gooch crucible, washed with 100 c.c. of 10 per cent. hydrochloric acid and dried at 100° C. for four hours. The amount of furfural can then be calculated from tables experimentally worked out and published by the authors in the above paper.

The analysis was only carried out in one experiment, and gave 14·4 per cent. of furfural, in that sample of mowrin.

The complete figures for this sample were as follows:—

Moisture	=	3·1 % (dried at 105° C.)
Furfural	=	14·4
Dextrose	=	41·2
Mowric acid	=	40·1
Total	=	98·8

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It was naturally expected from the foregoing results that mowrin would possess a very high molecular weight, and show no appreciable depression of the freezing point. For the molecular weight of the mowric acid lies at the lowest computation from its neutralization figure at about 600, and the brominated acid leads to a molecular weight of about four

1. *Zeitsch. f. angewandte Chemie*, Vol. XV, p. 477, 1902.

times this, that is, 2400, and the mowric acid forms only about 40 per cent. of the mowrin, so that, on the assumption of one molecule of mowric acid in one molecule of mowrin, the molecular weight comes approximately to about 6000.

We were, therefore, considerably surprised to find that the Δ of aqueous solutions leads to a molecular weight of only about 310 to 385 for the mowrin. Such a figure on the basis of 40 per cent. of mowric acid in the molecule would lead to a molecular weight of about 120 for the mowric acid.

An attempt to determine the molecular weight in glacial acetic acid solution demonstrated that so far from depressing the freezing point, the addition of the glucoside actually raised the freezing point, just as would an acid of higher melting point.

Experiment I.—A 5 per cent. solution of mowrin in distilled water was prepared, and the freezing points of this solution, and of the distilled water used in its preparation, were determined with a Beckmann apparatus as follows:—

Distilled water F.P.		Mowrin 5 % solution F.P.
2·659	...	2·340
	...	2·332
2·650	...	2·328
2·650	...	2·334
2·650	...	2·342
	...	2·336
2·650		14·012 $\div$ 6 = 2·335

$$\Delta = 2·650 - 2·335 = 0·315$$

$$\therefore \text{Mol. Wt.} = 19 \times 5 \div 0·315 = 302$$

Experiment II.—Molecular weight determination for a 5 per cent. solution of mowrin in distilled water.

Distilled water F.P.		Mowrin in 5 % solution, F.P.
3·518	...	3·280
3·516	...	3·276
3·521	...	3·276
		3·282
10·555		13·114
Average 3·518		Average 3·278

$$\Delta = 3·518 - 3·278 = 0·240^\circ \text{C.}$$

$$\therefore \text{Mol. Wt.} = 19 \times 5 \div 0·240 = 395$$

The lower molecular weight for the undecomposed sapo-glucoside is also suggested by the fact that it dialyses quite rapidly through parchment paper, which it would not probably do if it possessed the molecular weight of 6000 indicated for it by the bromo-acid.

The aqueous solution of the mowrin possesses also a fair amount of

electrical conductivity, which cannot be attributed to inorganic matter, as the substance leaves no ash, and contains nothing but carbon, hydrogen and oxygen. The reaction is neutral, so that there is no free organic acid or base present. This peculiar conductivity, therefore, is an enigma to us at present, just as is the apparently low molecular weight in aqueous solution.

The resistance of a 5 per cent. solution of mowrin is about four times that of a N/10 solution of potassium chloride. The freezing point determinations giving about 350 as an average, this 5 per cent solution would be about an N/7 solution; and it is remarkable that an N/7 solution of a glucoside should possess anything like one quarter of the conductivity of a decinormal solution of potassium chloride.

SECTION B. GENERAL PHYSIOLOGICAL PROPERTIES

BY F. W. BAKER YOUNG AND BENJAMIN MOORE

The only previous reference we have been able to trace to physiological or toxic properties is contained in Watt's work already quoted. Under the heading of Medicinal Properties he states that 'the cake left after the expression of the oil is employed as an emetic and as a detergent. It is used to poison fish, and the smoke produced in burning the cake is reported to kill insects and rats.'

In 1908, one of us was called upon to attend numerous cases of cellulitis of the hands and feet of a particularly virulent type, and found in every case that the patients themselves considered their condition to be due to the fact that they were working with the meal known as Mowrah meal.

In these cases, the characteristics observed were acute inflammation at the site of entry of the Mowrah meal, supervening in a few hours from any injury received. There was intense pain, and although suppuration was slight, there was considerable destruction of tissue. In several cases the inflammation attacked the periosteum, causing necrosis in one or more phalanges.

Men working with the meal complain of headache, occasional vomiting, watering of the eyes, and haemorrhage from the nose. There seems to be a good deal of personal idiosyncrasy as to the effects of the drug. An aqueous solution of the meal approximately of one in five strength was made, and 1 c.c. of this was injected subcutaneously in a rat.

Within five minutes there was evident paralysis of the hind limbs. The respirations, which at first had been rapid, became deep, slow, and heaving, and there was evident 'air hunger.' Death occurred in about a quarter of an hour from respiratory failure. On examination after death, the peritoneal cavity was found to contain blood-stained serum; the intestinal area was injected with blood, and blood was observed in the lumen. No haemorrhages were found in the stomach, but the whole small intestine was covered over with haemorrhagic areas, and there was great destruction and sloughing of the mucous membrane and epithelium. An examination of brain and cord showed a congested condition of the meningeal linings.

Death followed from a similar subcutaneous dose in frogs and in rabbits, and in the latter similar intestinal lesions were observed.

In marked contrast with the above are the effects of feeding by the mouth. Considerable amounts, much larger than the above, and as great as the animals would eat, were given mixed with other food, such as in bran to rabbits, mixed with rice to rats, and enclosed in meat to dogs, without any noticeable injurious effects. The animals appeared to remain quite well. The pure glucoside in big doses was also given enclosed in meat to dogs without any observable deleterious effects.

The lethal dose for the rat in subcutaneous injection was determined by the following experiments, as well as the symptoms and effects of the drug. The pure sapo-glucoside was used for the injections. Subcutaneous injection of 1 c.c. of 2 per cent. solution of mowrin in normal saline (= 0.020 gram) into a rat weighing 119 grams, caused death in one hour twenty-four minutes. The first symptom observable was paralysis of the hind limbs; the breathing then became slow and laboured, followed by inspiratory gasping and finally stoppage of respiration; the heart still beat for some little time after cessation of respiration, and it was found at the post-mortem, engorged on the right side, as usual in an asphyxial death.

A careful examination was made about half an hour after death. A transparent fluid was found in considerable quantity in both the abdominal and thoracic cavities, deeply stained with free haemoglobin. The intestines were dark coloured and injected. The right heart, as above stated, was engorged with black coloured blood, and on opening contained a dark-coloured clot. The whole of the inner surface of the small intestine was closely covered over by haemorrhages.

A subcutaneous injection of half the above dose (i.e., 0.5 c.c. of 2 per

cent. solution = 0.01 gram) was given to a rat weighing 129 grams. Paralysis of the hind limbs first set in, followed by that of the fore limbs, and at the end of about forty minutes the animal lay upon one side, showing strong dyspnoeic respirations. The dyspnoeic condition gradually increased in intensity, and the animal died of respiratory failure at the end of two hours thirty-three minutes. An autopsy showed a similar condition to that described above in the case of the first animal.

The dose was halved again, and tested also subcutaneously in a rat of 150 grams, dosage now being 0.005 gram given in 1 per cent. solution. Death occurred with the same symptoms and appearances in three hours five minutes.

In a fourth rat, the dose was again halved, now amounting to 0.0025 gram to a body-weight of 124 grams. This dose caused paralysis and dyspnoea, but the rat recovered from the first effects, and next day was apparently all right. It died, however, next day, but did not show the above appearances of mowrah poisoning. No marked appearances of inflammation were noticeable in the intestinal area, and no haemorrhages. The lungs were collapsed, only just floated in water, and showed red hepatization. They were filled with a red sanguinous fluid. The animal appears to us to have succumbed to a pneumonic infection, perhaps introduced by the lowering action of the drug.

This dose of 0.0025 gram in a rat of 124 grams hence appears to be about the minimal lethal dose on subcutaneous injection of mowrin in this animal.

The pure glucoside administered by the mouth in much larger quantities along with food produced, as already stated, no noticeable effects. It was also given enclosed in meat, up to as much as 0.5 gram in a dog of 7.5 kilos., without producing any obvious effect. No sugar appeared in the urine, nor could any mowrin be detected in it by chemical testing. The heart beat and respiration remained unaltered as far as we could observe.

The peripheral effect upon the blood vessels, as shown by the perfusion of the blood vessels in saline in the pithed frog, is shown in the accompanying tracing (Fig. 1), kindly taken for us by Messrs. Foster, Jones & Wright. The effect is a marked constriction, and this result was confirmed in several concordant experiments. The lowering effect upon arterial blood pressure on intravenous injection in the intact anaesthetized animal about to be described cannot accordingly be set down either to direct peripheral action or, as seen on the blood pressure tracing itself, to decreased heart activity.

On intravenous injection in an anaesthetized dog, the drug causes a distinct fall in blood pressure (see Fig. 2), with such a dose as we gave there was no apparent action on the heart, although in the isolated and perfused heart the striking results recorded in Section D were obtained. The explanation of this apparent anomaly appears to us to be that in the intact animal the mowrin is seized and bound, and so rendered inert by serum proteins, and the lecithides of corpuscles and tissues, so that little or none is left over to attack the heart lecithides and produce the characteristic results. On the isolated heart perfused with saline there are no other substances save the heart lecithides and heart proteins present to meet the attack of the mowrin. It accordingly enters into union, probably of a molecular or adsorption type, with these bodies altering their biochemical balance, and so interfering with the heart-beat. In any case, on direct introduction into the circulating blood there is none of that profound action on the heart which might perhaps have been expected *a priori*, from the known action on the isolated and perfused heart.

Such a difference in action on the isolated heart and on the heart *in situ*, renders the former a more exquisite test for physiological action of a cardiac drug; but it should also be remembered, in relationship to dosage, that a drug is going to produce many-fold less action in the heart *in situ* than an equal amount produces upon a heart excised and perfused with saline. A similar action occurring entirely *in vitro*, is seen in the difference in laking power of haemolytic agents (such, for example, as these very sapo-glucosides) (*a*) upon corpuscles *in serum* and (*b*) upon corpuscles *in saline*. In the latter case one-hundredth part or less of the haemolytic will produce as great an effect as in the former.

We have here accordingly to distinguish between a good and delicate engine for research, and the natural production of physiological, pharmacological, or pathological effects.

We believe that in this difference in equilibrium as between saline and blood, in the case of such organic colloidal or semi-colloidal bodies which can unite specifically with proteins or lecithides, lies the cause of apparently conflicting results as between excision perfusion methods and testing with the heart *in situ*.

In the case of mowrin, for example, if the exceedingly minute trace in saline which alters the heart-beat be taken as a basis, and the dosage calculated either upon volume of blood or body-weight which should produce a similar effect in the body, a very much sub-minimal dose will be obtained. Also, we know from our injection experiments with mowrin

FIG. 1.—Rate of flow in peripheral blood vessels before and after Mowrin shown by outflow through the Schäferfilter. The oscillations indicate outflows of 8 c.c. for each double oscillation shown electrically by upset of an oscillating trough (the Schäferfilter). The concentration of the solution perfused was 1 in 100,000 of Mowrin. The animal used was a pithed frog perfused through aorta, outflow from *sinus venosus*.

FIG. 2.—EFFECTS OF INTRAVENOUS INJECTION UPON ARTERIAL BLOOD PRESSURE.

Effects on arterial blood pressure of injection of 0.5 c.c. of 5 per cent. Mowrin (= 0.025 grams.) in dog of 5 kilograms. Anaesthetized by chloroform, cannulae in carotid artery and a crural vein—no other operative interference.

that death ensues from effects on the central nervous system; for death is preceded by paralysis and respiratory failure. A higher concentration is required to seriously affect the heart. Still, no better piece of physiological machinery than the isolated heart could be desired in order to demonstrate that the sapo-glucoside is toxic for cardiac muscle and to show the effects (see Figs. 4, 5, 7, 8 and 9, Section D) varying in quantitative amount with increasing dosage. As shown by Waller, skeletal muscle can similarly be employed as a pharmacological testing machine, and is somewhat simpler in its preparation. Here also the same remarks apply as in the case of the isolated heart, viz., that effective amounts, when dissolved in saline, will be obtained with much lower amounts than in natural perfusion in the blood; and hence that a marked effect upon the selected isolated tissue must not be taken to mean that that tissue will be the first to be attacked on exhibition of the drug in the intact animal.

SECTION C. HAEMOLYTIC ACTION

BY BENJAMIN MOORE

The action of mowrin, mowric acid, and bromo-mowric acid as haemolytic agents, has already been alluded to in a previous paper by Moore, Wilson and Hutchinson,¹ who found potent haemolytic effects with the sapo-glucoside, and also with its acid in considerably lessened degree, but scarcely any effect with the saturated bromo-compound.

Such haemolytic action is always found in all the sapo-glucosides in somewhat varying degree. It is similar in origin to that detergent action upon fatty or greasy stains, which has made them useful commercially as soap substitutes, and in the so-called dry cleaning of silken fabrics. This action they share with ordinary soaps, where the activity is chiefly due to oleates, and with the bile where the bile salts are the active constituents.

As we have already said, it is closely correlated with the action upon the heart, and the common cause of all this type of action, in soaps, bile salts, and sapo-glucosides is an adsorbing or molecular combining power for phosphatides or lecithides.

When union occurs between sapo-glucoside and erythrocyte lecithin, the result is haemolysis; when union occurs between sapo-glucoside and cardiac-tissue lecithin, the result is cardiac slowing, accompanied by

1. This Journal, Vol. IV, p. 346, 1909.

augmentation of the force of each beat with minimal or therapeutic doses, or by complete cessation in systole with larger doses. Although it has been proven by Moore and Roaf¹ that the anaesthetics such as chloroform and, to a much less degree, ether have similar affinities for proteins, it is not improbable that part of the action upon the heart tissue of anaesthetics may be due to a similar molecular attraction between lecithides and anaesthetics.

I desire, however, most distinctly and emphatically to point out that this is quite different from the Overton and Meyer view that the lecithides act as membranes to the cell of varying penetrability, and passable only to substances soluble in lecithides (so-called lipoids).

I have elsewhere shown that the lipoid membrane hypothesis breaks down both theoretically and practically, and while admitting both the importance and universality of lecithides in all cells, claim and definitely state that this is due *not* to their forming a membrane, surrounding or enclosing or forming a stroma to the other cellular constituents, but to their forming, along with protein and other organic substances, an integral and internal part of the living structure or bioplasm.

The action of drugs, such as the anaesthetics, or the sapo-glucosides with which we are here more intimately concerned, is hence a direct one upon the bioplasmic molecule, and not one due to interference with a membrane or the permeabilities of such membrane. In small doses, the haemolytic and cardiac drugs attach themselves to the bioplasmic aggregate (probably in part to the protein and in part to the lecithide) and by causing partial or total interlocking stop its physiological activity. In higher dosage, they decompose the bioplasmic aggregate altogether, disrupt it and carry off its lecithide. This leads to disruption of the entire cell such as we see in haemolysis.

Such a view has a firm basis in experimental facts, and correlates similar actions of drugs and substances which are at first sight quite dissimilar.

It correlates, for example, and explains upon a common basis, the laking of blood corpuscles by organic solvents such as ether or chloroform, with the similar action of the soaps, bile salts and sapo-glucosides; and places this set of phenomena in line with the similar action of all these bodies upon the heart. All this group, in varying quantitative degree, slow the heart-beat, lower arterial blood pressure, and lake blood corpuscles.

1. *Proc. Roy. Soc., B.*, Vol. LXXXVII., p. 86.

Mowrin, as shown by the subjoined protocols, is an exceedingly powerful haemolytic. As will be observed, it shows no trace of that peculiar exaggerated action of lower concentrations discovered in this laboratory by McLean and Hutchinson¹ for certain bile salts and their derivatives, and called by them the *haemolytic paradox*. Instead of showing this the mowrin solutions show increasing haemolytic power, with increasing concentration, from doses which show no action up to doses which completely lye in ten seconds and are present in 7.68 per cent. concentration. The effect is continuous, and there is nowhere any break in the curve.²

This does not, however, mean a fundamental difference between bile salts and saponin-glucosides, for we are at present in this laboratory engaged upon a study of the whole group of saponin-glucosides, and we have found in *Hederin*, a saponin-glucoside of the leaves of Common Ivy (*Hedera helix*), an exquisite example of a haemolytic agent showing the paradox. The laking with very low concentrations being almost instantaneous and the time of laking increasing with increasing concentration of haemolyser.

The results of this investigation will shortly be published in a separate paper; they are mentioned here to indicate that the haemolytic paradox is shown by some haemolysers and not by others.

Haemolytic experiments. The experiments were carried out in the usual fashion in tubes containing a total volume of 5 c.c., made up of 1 c.c. of twice washed red blood corpuscles of the sheep made up to a 5 per cent. emulsion in 0.9 per cent. normal saline; plus a variable amount of a definite concentration of the mowrin dissolved in 0.9 per cent. normal saline; plus, again, a sufficient amount of 0.9 per cent. saline to make up to 5 c.c. total volume.

The experiments were carried out at ordinary laboratory temperature. The times count from final making up of the tubes, and were controlled by two observers. The first table shows the high degree of haemolytic power possessed by mowrin, and the absence of the paradox; the second table shows a balancing action against pig's serum which, as is well known, is haemolytic for sheep's red blood corpuscles.

The two haemolysers, instead of showing an additive action, retard each other. In other cases, two given haemolysers aid each other and show a purely additive effect, such, for example, as sodium oleate and sodium linoleate acting together.

1. *Bio-Chemical Journal*, Vol. IV, p. 369, 1909.

2. In a later experiment on the haemolytic powers of the sodium salt of the isolated mowric acid, the interesting fact came out that this body does not show any paradox when freshly made, the haemolytic power increasing continuously with the concentration; but if the solution is preserved stoppered up for about a fortnight, the paradox appears in a marked degree, and at the same time the solutions become more highly active. This change is probably due to chemical polymerization, and is being investigated further.

Mowrin behaves like oleates, linoleates, and bile salts in retarding pig's serum. It is therefore probable that mowrin would act reinforcingly in presence of these simpler haemolytics, and like them would balance against lecithides.

The results obtained are shown in the two Tables on pp. 114 and 115 and in Fig. 3, where the results of a different experiment are graphically plotted.

FIG. 3.—HAEMOLYTIC ACTION OF MOWRIN IN MILLIGRAMS.

I have to express my thanks to Messrs H. V. Foster, H. W. Jones and S. N. Wright, research students in this laboratory, who have rendered me much able assistance in carrying out the experiments of this Section.

TABLE I. HAEMOLYTIC POWER OF MOWRIN, AND ABSENCE OF HAEMOLYTIC PARADOX

Tube No.	Amount of mowrin solution in normal saline	Corresponding weight of mowrin in grams in the 5 c.c. of solution	Percentage of mowrin in tube when completely made up	Volume of normal saline added to make up to 5 c.c.	Result : Complete haemolysis in—
I ...	4 c.c. of 9·6%	0·384 gram.	7·68 %	Nil	10 sec.
II ...	2 „ „	0·192 „	3·84 „	2 c.c.	15 „
III ...	1·6 „ „	0·154 „	3·08 „	2·4 „	18 „
IV ...	1·2 „ „	0·115 „	2·30 „	2·8 „	20 „
V ...	0·9 „ „	0·086 „	1·72 „	3·1 „	23 „
VI ...	0·5 „ „	0·048 „	0·96 „	3·5 „	28 „
VII ...	4 c.c. of 0·6 %	0·024 gram.	0·48 %	Nil	30 sec.
VIII ...	2 „ „	0·012 „	0·24 „	2 c.c.	33 „
IX ...	1 „ „	0·006 „	0·12 „	3 „	50 „
X ...	0·5 „ „	0·003 „	0·06 „	3·5 „	1 min. 40 sec.
XI ...	0·25 „ „	0·0015 „	0·03 „	3·75 „	5 „ 47 „
XII ...	2 c.c. of 0·0375 % solution	0·00075 gram.	0·015 %	2 c.c.	8 min.
XIII ...	1 „ „	0·000375 „	0·0075 „	3 „	18½ „
	0·5 „ „	0·000187 „	0·0037 „	3·5 „	60 „
XIV to XVII }	... Contained still lower traces of mowrin, varying from 0·001 to 0·0001 %, and showed no haemolytic effect within four hours.				
One c.c. of a 5 % emulsion of washed sheep's red blood corpuscles in 0·9 % saline always added.					

TABLE II. The balancing action of *mowrin* against *pig's serum* in the haemolysis of *sheep's red blood corpuscles*:—

Set I	5 % emulsion of sheep's red blood corpuscles	0.1 % solution of mowrin in normal saline	Pig's serum	Saline	Calculated weight of mowrin used	Times for complete haemolysis
No. 1	1 c.c.	2.0 c.c.	Nil	2.0 c.c.	0.002 gram.	10½ min.
„ 2	1 „	1.5 „	„	2.5 „	0.0015 „	15½ „
„ 3	1 „	1.0 „	„	3.0 „	0.001 „	23½ „
„ 4	1 „	0.75 „	„	3.25 „	0.00075 „	38½ „
„ 5	1 „	0.5 „	„	3.5 „	0.0005 „	1 hr. 37 min.

This series was placed in the incubator at 27° C. 3½ minutes after mowrin had been added to tube No. 1.

Set II

Serum alone	1 c.c.	Nil	1 c.c.	3 c.c.	—	20 min.
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Set III

Placed in incubator immediately.

No. 1	1 c.c.	2 c.c.	1 c.c.	1.0 c.c.	0.002 gram.	16 min.
„ 2	1 „	1.5 „	1 „	1.5 „	0.0015 „	19½ „
„ 3	1 „	1.0 „	1 „	2.0 „	0.001 „	29 „
„ 4	1 „	0.75 „	1 „	2.25 „	0.00075 „	45½ „
„ 5	1 „	0.5 „	1 „	2.5 „	0.0005 „	40 „
„ 6	1 „	0.25 „	1 „	2.75 „	0.00025 „	21 „

This series was placed in the incubator about 3 minutes after addition of haemolysers to the first tube.

Control ...	1 c.c.	—	—	4 c.c.	—	Unchanged after 5 hours
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The notable point is that at No. 4, Set III, 0.75 c.c. of 0.1 % mowrin, plus 1 c.c. of pig's serum, require 45½ minutes to produce complete laking, while an equal concentration of mowrin lakes in 38½ minutes, and an equal concentration of pig's serum lakes in 20 minutes.

SECTION D. ACTION UPON THE ISOLATED MAMMALIAN
HEART

By S. C. M. SOWTON

The method of perfusing an isolated heart through its coronary vessels is too well known to need description. The tests were made upon the hearts of rabbits, the animals used being from six to nine months old. The fluid used for perfusion was Locke's modification of Ringer's solution (without the sugar) supplied under constant oxygen pressure. Two flasks of the fluid were prepared, to one of which was added a given amount of the substance to be tested; the heart was allowed to settle down into a steady rhythm, and when this had continued for some time the drugged fluid was substituted for the normal Ringer's solution and the flow of it was continued throughout the experiment. The heart-beats were recorded in the usual way.

An orientating experiment (Fig. 4) with the glucoside at a strength of 1 in 2000 resulted in the arrest of the ventricle in four and a half minutes. The record of this very short experiment is not without interest, as it gives in a compressed form effects afterwards more conveniently observed with weaker solutions of mowrin and of mowric acid. The tracing shows the contractions increasing in size, then diminishing as the toxic action of the glucoside takes effect, this latter phase being accompanied by the final rise in tonus, which is so characteristic an end-effect with drugs belonging to the digitalis group, when used in a similar manner. Upon the coronary vessels the effect of mowrin at this strength was constriction so great as almost to arrest the flow of fluid.¹ A similar effect has occasionally been obtained with certain tinctures of digitalis when used at high percentages.

The next illustration (Fig. 5) is from the record of an experiment with mowrin much more diluted, namely, at 1 in 100,000. Slowing in the rate of beat with increase in its size is to be noticed, while rise of tonus becomes very marked as the experiment proceeds. At intervening strengths the glucoside gave similar results, but as these experiments form a less complete series than those made with mowric acid, attention is drawn more particularly to the latter experiments. They were ten in number, and were made from one 'brew' of the acid, whereas in the case

1. In Section B, p. 107 (see also fig. 1), it is shown that a similar constriction is produced in the peripheral blood vessels when dilute mowrin in Ringer's solution is perfused through the vessels.

of the glucoside two preparations were used, and they may have differed slightly.

For convenience of comparison, the record of a digitalis experiment is given in Fig. 6; it shows the well-known effects. The normal rate of beat, which was 152 per minute, is reduced throughout¹ and increase in amplitude of contraction is evident during the first half of the experiment. Later, tonus rises, and at the end of twenty-six minutes the ventricle is arrested in a state of tonic contraction. The tincture was used at a strength of 1 in 200. The highest strength at which mowric acid was used was 1 in 5000 (Fig. 7); as compared with the digitalis tracing, the similarity of effect is sufficiently striking; the normal beat is slowed during the first part of the experiment and the amplitude of contraction increased. Later, tonus rises gradually, till, at the end of twenty-six minutes, the ventricle is arrested in tonic contraction.

The effect of mowric acid at 1 in 10,000 is shown by Fig. 8. The normal rate of beat of 136 per minute is gradually slowed; in twenty-five minutes it is 100 per minute; in fifty-three minutes it has fallen to 86 per minute; while still later the fall has reached 76 per minute, a reduction of 44 per cent.; this was the most marked reduction observed in any experiment. Increase in amplitude of beat is well shown, expansion as well as contraction being more complete, and tonus rises during the latter part of the experiment. In another experiment at this strength the reduction in rate of beat amounted to 29 per cent., and the size of contraction was increased by a third. Experiments were also made with the acid at 1 in 20,000 and 1 in 50,000; the slowing was less pronounced, but the increased size of contraction was always very well marked. In some cases this increase was preceded by a short-lasting diminution, such as is sometimes seen to follow the exhibition of the digitalis drugs.

In Fig. 9 the effect of the acid at 1 in 100,000 is shown; the slowing in rate and augmentation in size of beat are both evident. The experiment lasted one hour ten minutes and shows only the earlier phase of activity of the acid, that which in speaking of digitalis action would be called the 'therapeutic stage.'

At 1 in 200,000, the lowest strength used, the limit of activity of mowric acid appeared to be nearly reached; we have, however, but a single experiment at this strength. Some slight slowing was produced during the hour that the experiment lasted, at the end of which time the solution was exhausted.

1. With many tinctures of digitalis, and with all, so far as we know, of squill and strophanthus, the period of rising tonus is accompanied by a quickened beat, and the heart tends to pass into the condition of delirium.

Omitting the last experiment in which the slowing effect was very slight, the reduction in rate of beat, taking the average of nine experiments, amounted to 21 per cent. This is below the average diminution observed for tincture of digitalis, as given by Haynes,¹ who puts it at 30 per cent., the tincture being used at constant strength. In the present experiments, however, the strength of mowric acid varied, as has been seen, from 1 in 5,000 to 1 in 100,000, and a strict comparison cannot, therefore, be drawn.

The augmentation in size of contraction was in these experiments very marked, the average increase amounted to 64 per cent.; in three cases it was over 100 per cent. It was more marked with the acid than with the glucoside.

It has thus been shown that the action of mowric acid upon the isolated heart resembles that of the drugs of the digitalis group, alike in the earlier or 'therapeutic stage,' and in that later phase which is marked by a rising tonus. It is during this last stage of activity that irregularities of rhythm occur when the digitalis-like drugs are used, and the irregularities observable are of two types: that characterised by a dropping out of ventricular beats, and the more profound disorganisation of rhythm known as delirium cordis. The dropping of ventricular beats was observed when mowric acid was used at high strengths; it occurred in the two experiments at 1 in 5,000 and in one of those at 1 in 10,000. Delirium cordis did not occur in any experiment made with the acid, and one instance of it only was observed in the experiments with mowrin. In respect of their disorganising properties, therefore, the mowrah preparations would appear to resemble digitalis more nearly than they resemble squill or strophanthus. Strophanthus used at a strength sufficient to stop the beat in four and a half minutes, as is done by mowrin (Fig. 1), would, it may be said almost with certainty, have given the final quickening of beat which ends in delirium. Such an effect is much less certain with digitalis, many tinctures of which, as has been pointed out elsewhere,² can be used at high strengths without giving any trace of delirium.

In respect of strength, mowric acid is very much stronger than tincture of digitalis; how much stronger, the experiments so far made are not numerous enough to determine with any exactness. But judging by such indications as are available, one would say, roughly speaking, that the acid is some twenty-five times as strong as a good tincture. Taking

1. 'Pharmacological Action of Digitalis,' etc., this Journal, Vol. I, No. 2, p. 69.

2. 'Some Experiences in the Testing of Tincture of Digitalis,' *British Medical Journal*, February 6, 1908.

as a criterion of strength the time required to stop the ventricular beat, it is found that with mowric acid at 1 in 5,000 the mean of the time of arrest in two experiments was twenty-two minutes. In the experiment with digitalis at 1 in 200 (Fig. 3) the ventricle was stopped in twenty-six minutes, which is just above the average time of arrest for that strength of the drug as calculated from a large number of experiments.

In some of the experiments under consideration, though not in all, the heart became more oedematous than is usual in similar perfusions with the digitalis drugs; that at least is the impression derived from direct inspection of the heart.

FIG. 4. RABBIT'S HEART PERFUSED WITH RINGER'S SOLUTION

Effect of Mowrin at 1 in 2000. The ventricle is arrested in four and a half minutes, in tonic contraction.

In this and in the following figures the line immediately below the heart tracing is the electric record of the movements of the 'Schäfer tilter' by means of which the rate of flow can be measured. In this particular tracing the constricting effect is so great that the flow is almost arrested. The oscillations are well seen in the succeeding figures. The lowest line marks the time at intervals of 5 seconds.

FIG. 5. RABBIT'S HEART PERFUSED WITH RINGER'S SOLUTION

Effect of Mowrin at 1 in 100,000. The beat is reduced in rate and increased in size. The ventricle is stopped ; a state of strong tonus.

FIG. 6. RABBIT'S HEART PERFUSED WITH RINGER'S SOLUTION

Effect of Tinct. Digitalis at 1 in 200. A piece of normal tracing is first shown; the succeeding portions of the record show the effect of the drug at intervals during the course of the experiment; in the last portion, twenty-three minutes after onset of the drug, the beat is only just visible and tonus is very high.

FIG. 7. RABBIT'S HEART PERFUSED WITH RINGER'S SOLUTION

Effect of Mowric acid at 1 in 5,000. Slowing of beat is shown, and increase in force of contraction.

* At this part of the experiment the auricle was giving two beats to one beat of the ventricle; later there were several auricular beats to one ventricular beat. The ventricle is arrested in a state of strong tonus.

FIG. 8. RABBIT'S HEART PERFUSED WITH RINGER'S SOLUTION

Effect of Mowric acid at 1 in 10,000. Slowing in rate of beat and increase in force of contraction is shown. Tonus rises in the latter part of the experiment. The ventricle is arrested at the end of two hours forty minutes.

FIG. 9. RABBIT'S HEART PERFUSED WITH RINGER'S SOLUTION

Effect of Mowrah acid at 1 in 100,000. The beat is reduced in rate and increased in size. The experiment was stopped in one hour ten minutes, and shows only the earlier effects of the drug.