STUDIES ON THE TOXICITY OF BILE.

I.

THE EFFECTS OF INTRAVENOUS INJECTIONS OF BILE UPON BLOOD PRESSURE.

By S. J. MELTZER, M.D., and WILLIAM SALANT, M.D., of New York.

(*From the Rockefeller Institute for Medical Research, New York.*)

Plates XXIII–XXVI

In a recent communication by Arthur Edmunds,[1] it is stated that the fall of blood pressure produced by intravenous injection of bile salts is insignificant. The statement is supported by the reproduction of tracings obtained from such experiments. The one obtained after an injection of 5 cubic centimeters of a 1 per cent. solution of sodium glycocholate shows hardly any depression. The depression seen on the tracing obtained from an injection of 5 cubic centimeters of a 1 per cent. solution of sodium taurocholate corresponds to a fall of not more than 15 or 20 millimeters of mercury. The author states further that the subject was investigated in the same laboratory [2] a few years before by Dr. A. Young, who also found that the effect of bile salts on blood pressure was very slight. With 5 per cent. solutions of the salts the fall observed by him was hardly greater than that shown in the figures presented by the present author.

There have not been very many studies of the effect of bile upon the blood pressure, and of those made only few workers have given particulars or reproduced kymographic tracings. L. Traube,[3] who was the first one to study the blood-pressure effect of these salts by means of the kymograph, states that an intravenous

[1] Arthur Edmunds, *Brit. Med. Journal*, 1905, i, 57.

[2] Physiological Laboratory of King's College, London.

[3] L. Traube, *Berl. klin. Wochenschrift*, 1864, i, 85.

injection of 4 to 6 cubic centimeters of a 33 per cent. solution of "Natrium choleinicum" produces a considerable lowering of the blood pressure, but he fails to give figures or to reproduce tracings presenting the character and the degree of the reduction of the pressure. M. Loewit,[4] who injected sodium cholate intravenously, states that the effect upon blood pressure is variable, depending upon the dose injected as well as upon the individuality of the animal. Besides Edmunds, Loewit is, as far as we know, the only one who published tracings presenting the lowering of the blood pressure, and the strongest effects which the latter author obtained were apparently not greater than those which were obtained by Edmunds. There are a few other writers who have reported the lowering effect of bile salts upon the blood pressure (Landois, Röhrig, Feltz and Ritter, and others), but their statements are not precise enough to permit a comparison of the degrees of the depressing effect. We have to add that nearly all the authors who studied the blood pressure confined their investigations exclusively to the effects of bile salts. There is nowhere any definite statement as to the effect of pure bile upon the blood pressure.

The results to be presented here were derived nearly exclusively from experiments made by intravenous injections of filtered ox bile or solutions of inspissated ox bile. We have chosen the whole bile in the first place for economic reasons, as taurocholic and glycocholic acids are too expensive for use in considerable quantities in a large number of experiments. But we wish also to record the statement that we have reason to doubt the validity of the now nearly universal belief that all the physiological and toxic effects of bile are due to the bile salts alone. It may be considered as proven that neither the bile pigments nor cholesterin nor any other constituent of the bile is capable of producing effects similar to those produced by the bile salts. But it is far from being proven for bile, as in fact it is not proven for any biological product, that the effect of the sum of all hitherto known parts is equal to the effect of the whole. Our doubts as to the absolute identification of the effects of bile with the bile salts

[4] M. Loewit, *Zeitschrift für Heilkunde*, 1881, ii, 459.

arose in the course of another series of experiments on the toxicity of bile, and we shall therefore not enter here upon a discussion of the real merits of these doubts.

Our experiments were made on rabbits which were anæsthetized by ether. The injections of bile were made from a burette into the jugular vein and the blood pressure was taken from the carotid artery and transmitted by means of a mercury manometer to a rotating drum. The tubes connecting the artery with the manometer were filled with a solution of carbonate of soda or with a mixture of carbonate and bicarbonate of soda.[5] In some experiments tracheotomy was also performed so as to be in readiness for artificial respiration.

We may state at the outset that our experiments brought out often enough both extremes of action. Sometimes the injections of a good quantity of bile would exert only an "insignificant" influence upon the blood pressure, while at other times even a comparatively small dose of bile would produce a "considerable" fall of the pressure, and this occurred on the same animal and with the same concentration of the bile solution. However, these variations in the effects could be produced at will, for they depended largely upon the rate with which the bile was introduced into the circulation. We can therefore subscribe to the statement of Loewit that the dose of the injected bile exerts a distinct influence upon the degree of the effect, and admit also that the individuality of the animal may have something to do with the results. It might be added further that the concentration of the bile is a very important factor, and the general condition of the animal and the original height of the blood pressure, etc., are also factors in the reaction of the blood pressure to the introduction of bile. But all these factors together cannot compare with the importance of the rate of injection on the effect of the bile upon the blood pressure. Ten cubic centimeters and more of concentrated bile can be introduced into the veins of a rabbit without reducing the blood pressure by as much as 15 or 20 millimeters of mercury

[5] Under no circumstances should magnesium sulphate be used in the experiments. The reasons for this caution will be discussed in a future paper.

if only the injection be carried out slowly. On the other hand, even 2 or 3 cubic centimeters of a moderately concentrated solution of bile are capable of reducing the blood pressure by 60 or 70 millimeters when it is permitted to flow rapidly into the vein.

Furthermore, the rapid injection of even a small quantity of bile may have a fatal effect upon the rabbit. Thus the question of the rate of injection may serve not only to reconcile the divergent statements with regard to the influence of bile upon blood pressure, but it may also clear up the contradictions which abound in the literature regarding the fatal effects of an intravenous injection, at least so far as the immediate effects are concerned.

In the early part of the last century it was stated by Magendie and other investigators that an intravenous injection of bile is invariably fatal to the animal. However, the bile which was used was not filtered and it was later assumed that the death of the animal was due to emboli produced by the impurities of the bile. Bouisson reported that rabbits which received 6 cubic centimeters of filtered bile survived the injections. During the last fifty years numerous experiments were carried out with clear solutions of biliary salts, free from corpuscular and from muciginous elements. In the literature on this aspect of the subject we nevertheless meet the same puzzling conflicting experiences. Some authors, such as Frerichs, for instance, have never seen any fatal results from the injection of bile except when air entered the veins. Others, like Leyden, Von Dusch, etc., lost at times some of their animals immediately after an injection of only 1 cubic centimeter of a 10 per cent. solution of bile salts. Von Dusch lost some of his rabbits two or three minutes after injecting a few cubic centimeters of filtered ox bile.

Judging from our experience we have reason to believe that all these contradictions have their chief origin in the variation of the rate with which the injections were made. Six to seven cubic centimeters of bile seem to have been the largest quantity which was ever injected into a rabbit. We have injected into

one rabbit as much as 20 cubic centimeters of a 50 per cent. solution of ox bile within forty-four minutes, and not only was the rabbit apparently in a normal state at the conclusion of the injection but the circulation was even improved; the blood pressure having risen from 100 millimeters of mercury at the beginning to 118 millimeters at the end of the experiment. By extending the injection over a longer period, we have in another experiment injected 50 cubic centimeters of a 50 per cent. solution of ox bile without any visible immediate detriment to the animal. On the other hand, a small dose of 2 or 3 cubic centimeters of bile sufficed to kill a rabbit almost instantaneously when injected very rapidly. We have made, intentionally and unintentionally, a number of such experiments. The blood pressure would sink rapidly to so low a level that even immediately instituted artificial respiration failed to restore it. (Fig. 1 and Fig. 5, Plate XXIII.) The animals died frequently while under respiratory convulsions, which explains the statement of some writers that intravenous injections of bile often cause immediate convulsions.

That the rapidity with which the injections are carried out is an important factor in the results obtained is of course not a new idea. It has often been mentioned by writers dealing with the effects of injections of active substances, and was especially mentioned in connection with the recent studies on the toxicity of urine. But this factor has not been mentioned, at least as far as we know, by writers dealing with the effects of injection of bile upon the circulation. We missed especially allusion to it in the article of Edmunds, since in dealing there with the effects of potassium chloride the tracing given shows that the quantity of the salt which when injected within eight seconds caused only a moderate depression, caused when injected within two seconds a fatal fall of the blood pressure.

That the considerable fall of blood pressure which is caused by a rapid injection of bile is due to the toxicity of the bile and not to the mechanical effect of the injection we have proved by rapidly injecting a solution of 0.9 per cent. sodium chloride. An injection of 10 cubic centimeters or more of sodium chloride

in 20 or 30 seconds marks hardly any change on the blood-pressure curve, while less than half this amount of bile injected within a similarly short time is surely fatal to the animal. In Fig. 2, Plate XXIII, at 1, 10 cubic centimeters of sodium chloride were injected in 10 seconds, and at 2, the same amount of the normal salt solution was injected within 20 seconds. In both cases there was scarcely any effect. Eight minutes later, 4.5 cubic centimeters of pure bile (at 3) were injected within 30 seconds, and the blood pressure sank rapidly, the animal dying within 3 minutes after the injection.

The same experiment also serves to demonstrate that the fall in blood pressure is not due to the low temperature of the bile, since the salt solution as well as the bile was injected at room temperature.

An analysis of our results brings out instructive details. The suddenness with which the blood pressure falls and the steepness of the descending branch in the blood-pressure curve depend essentially upon the rapidity of the injection. In rapid injections the fall of pressure sets in immediately at the beginning of the injection, and only exceptionally is it preceded by a very short slight rise (Fig. 2 at 3, Plate XXIII). The curve representing the fall is the steeper the more rapid the injection. If the injection was made more slowly either at the beginning or at the end, or at any phase, this fact appears well marked on the curve; and when after a considerable fall the injection was slowed, the pressure began to rise before the injection was finished (Fig. 4, Plate XXIII). In rapid non-fatal injections the blood pressure begins to rise immediately on cessation of the injection, and the ascending branch of the curve becomes for the most part a counterpart of the descending branch. In other words, the blood pressure rises at the same rate as it fell. Sometimes the pressure rises a little above the original level only to fall again immediately, for a short period, below this level. A momentary rapid rise to a point above the original height may occur even in cases of a fatal drop of blood pressure (Fig. 5, Plate XXIII).

If care be taken to avoid considerable depressions, rapid injections may often be repeated and kept up for a comparatively

long period without affecting the mean level of the pressure; indeed in some experiments the blood pressure was at the end higher than at the beginning.

When the injection is carried on slowly there may at first be no effect at all. Later the respiratory, Traube-Herring, and other waves of the normal blood-pressure curve disappear one after another and the blood pressure commences to descend slowly. When a slow injection is extended over many minutes, the blood pressure may become reduced by 50 or more per cent. However, the curve representing the fall is not throughout a straight line; the more it descends the more it becomes concave at its upper aspect, which shows that the depressing effect of the bile is gradually diminishing (Fig. 3, Plate XXIV). Nevertheless, even with slow injections, if continued too long, a fatal level from which recovery is no longer possible may be reached. When a slow injection is interrupted, the beginning of the return of the pressure is sometimes delayed a little; but when the return begins the ascending branch of the curve here too is nearly a counterpart of the descending branch; it rises at first slowly and then more rapidly, and the last result, as a rule, is the return of the waves. In slow injections, however, the return is not entirely complete, and the oftener the injection is repeated the lower the level of mean pressure becomes, so that at the end of such an experiment, even if not very prolonged, the blood pressure may be much lower than at the beginning. However, this result is met with chiefly in those experiments in which the injection extends over longer periods of time.

While the rapidity of the fall thus depends essentially upon the rapidity of the injection, the degree of the fall is greatly influenced by the concentration of the bile. For instance, 1 cubic centimeter of a 2 per cent. solution of ox bile injected within ten seconds will reduce the pressure only by 10 to 12 millimeters (see Fig. 7, Plate XXIV), whereas 1 cubic centimeter of undiluted bile injected at the same rate will reduce the pressure by 30 millimeters and more (Fig. 3, Plate XXIV), and 1 cubic centimeter of a 20 per cent. solution of inspissated ox bile injected within 60 seconds will reduce the pressure by 50 or 60 milli-

meters (Fig. 8, Plate XXIV), and the same solution injected in 20 seconds will surely be fatal to the animal (Figs. 1, 2, and 9, Plates XXIII and XXV).

These facts again show that the fall of blood pressure is due to the specific effects of the bile and not to mechanical causes or to the lower temperature of the injected fluids.

From a few observations we have made it would seem that the general condition of the animal and the original height of the blood pressure are factors also influencing the degree of fall produced by the injection of bile. Furthermore, in some cases the injection of bile caused immediately a slowing of the heart-beat, and in those cases the blood pressure reacted more readily and sometimes fell rapidly to a fatal level. In them the descending curve is very steep and shows only a few heart-beats (Figs. 1 and 5, Plate XXIII).

All writers agree that it is the detrimental effect of the bile upon the heart which causes the fall in the blood pressure, although there is disagreement as to whether the heart muscle, or the heart ganglia, or both, become affected by the bile. As regards the nature of the affection, Traube, Leyden, and others believe it to be malnutrition of the heart caused by the destructive action of the bile upon the blood corpuscles. There is no longer any doubt that bile is a strongly hæmolytic substance, but it is an open question whether this factor is especially active in causing the drop in blood pressure. Even at body temperature it takes some time for bile to produce perceptible hæmolysis, whereas the injection of bile into the circulation may produce a fatal drop of blood pressure in a few minutes. We have, however, disproven the hæmolytic theory by a direct experiment. By injecting rapidly a few cubic centimetres of bile, the blood pressure dropped quickly to a fatal level (Fig. 1, Plate XXIII) and the animal died in less than two minutes. The blood was immediately taken from the right ventricle and mixed with normal salt solution; no trace of hæmolysis could be discovered. It can be safely assumed, we believe, that hæmolysis has no share in the fall of blood pressure and the sudden death of the animal following immediately upon the injection of bile.

The autopsies performed immediately after such sudden death have not shown definitely the cause of death. In one case there was considerable pulmonary œdema, the lungs were very hyperæmic, and the right ventricle contained a clot. In most of the autopsies, however, the findings were negative except that both ventricles were distinctly dilated and flabby. The heart's blood was either fluid or was firmly clotted in both ventricles. The lungs were normal. The appearance of the heart was suggestive of paralysis; and if this actually existed it may be conceived either as being due to anatomical changes in the organ or as being the expression of a functional effect, such as inhibition upon the heart's action. As regards the anatomical changes, it has been shown through various investigations that bile alters the structure of muscle tissue as well as nerve fibres and nerve cells. It would, however, be surprising should anatomical changes, as in the fatal cases on the one hand, take place within a few seconds from the beginning of the injection, and as in the case of non-fatal effects, such a rapid structural restitution on the other hand, occur.

Regarding the inhibitory effect of the bile upon the heart, we wish to report some interesting experiments. Before introducing the bile in certain rabbits, one vagus (left) was cut and the minimum strength of electrical stimulus, which caused a distinct inhibitory effect by application to the peripheral end, was established. For the induction coil we used one Daniell cell, and its distance was 300 and 250 millimeters in the rabbits in which this experiment was carried on. A dose of bile, which caused a considerable fall of the blood pressure, was injected, and during the persistence of the fall the irritability of the peripheral end of the vagus was tested, and it was found that not only had the irritability not suffered but that the effect was stronger than before the injection; moreover, in two instances a stimulus which was ineffective before the injection became effective during the fall of the blood pressure (Figs. 8, 9, 11, and especially Fig. 10, Plates XXIV, XXV, and XXVI). We are not aware that such experiments were made before.

In still other experiments we have made the following in-

structive observations. Bile was introduced in such a manner as to cause a fatal fall of blood pressure and the peripheral end of the vagus was then stimulated. It was established that the inhibitory effect of the vagus remained intact as long as there were any signs of a heart-beat. Even when the pressure was not more than four millimeters and the heart-beat hardly perceptible, stimulation of the vagus caused a standstill of the heart (see especially Fig. 9, Plate XXV). O. Sokolow [6] and Loewit [7] state that vagus inhibition passes off before the heart stops. They have published no tracings and have given no data. Our tracings show that not only does the vagus inhibition last as long as the heart-beat, but that it retains its full irritability to the very end.

Our experiments further demonstrate that while the heart's action rapidly fails under the influence of bile, the inhibitory mechanism so far from being impaired by it may even be improved. This fact would favor the view that the effect of the bile upon the heart is a functional one, or, in other words, that bile stops the heart by inhibiting its activity. We do not mean that bile effects the inhibition through the vagus, because we know that the heart is stopped by bile even after eliminating the inhibitory effect of the vagus by a previous atropinization of the animal. Bile may, however, affect the heart in the same manner as potassium salts, the substances which contain three methyl ammonium groups, (Luchsinger and his pupils) and lactic acid (Gaskell [8]), which "weaken the contractions of the heart and ultimately bring it to a diastolic standstill." We see no reason why this action should not be called inhibition, brought about by some kind of a mechanism which is located in the muscle fibres or in invisible ganglia—whichever one chooses to assume. We may state further that we can see no objection to the assumption that the inhibition of the vagus is carried out through this mechanism and that atropin only interrupts the transmission of the vagus impulses through it. The latter

[6] Sokolow, *Physiol. u. toxicol. Studien am Herzen*, Inaug. Dissert., Bern, 1881.
[7] Loewit, *l. c.*
[8] Gaskell, *Schaefer's Text-Book of Physiology*, ii, p. 222.

assumption would, however, collide with the prevailing orthodox views on the subject upon which we do not wish here to enter into a discussion.

DESCRIPTION OF PLATES.

The tracings were obtained in the usual manner by connecting the carotid artery with a mercury manometer which transmitted the variations of the blood pressure upon a revolving drum covered with smoked paper. The curves read from left to right. Besides the blood-pressure curve, there were recorded on each figure a base line, a time-marking line mostly in four seconds, and sometimes also a line marking the beginning and the end of an injection. For lack of space these lines were omitted in most of the reproduced figures. The bile used was either filtered ox bile, pure or dilute, or dissolved inspissated ox bile.

Fig. 1. 2.5 c. c. of 10 % ox bile rapidly injected into the jugular vein, the injection finished in 4 seconds. The blood pressure immediately dropped deep down and the animal was dead in less than 2 minutes. The blood from the right ventricle showed no hæmolysis.

Fig. 2. At "1" 10 c. c. of 0.9 % sodium chloride were injected in 20 seconds; at "2" the same amount of the same solution was injected within 10 seconds; both injections produced scarcely any perceptible effects upon the blood pressure. At "3" 4.5 c. c. of pure bile were injected in 30 seconds. There was at the beginning a very short slight rise, then a rapid fall of the blood pressure followed. The pulse became slower and artificial respiration was instituted, but the animal died in less than 3 minutes.

Fig. 3. At "1" 1 c. c. of undiluted bile was injected in 30 seconds; there was hardly a perceptible effect upon the pressure; at "2" and "3" each 1 c. c. more was injected in 9 seconds, the falls produced were equal to 25 and 30 millimeters of mercury; a slight rise preceded the fall. At the last point of the fall the injection was finished, and immediately the pressure rose again in the same manner as it fell. At x in "4" the injection of 5 c. c. of bile began and lasted 4 minutes; at x at the bottom of the curve the injection was finished, the low pressure lasting a few seconds, after which the rise began and reached the former level. The end of the curve is not reproduced.

Fig. 4. 2.5 c. c. of undiluted bile injected in 40 seconds. The injection was made at first slowly, then more rapidly, and finally considerably more slowly. The pressure began to rise before the injection was finished. The different rates of injection are well shown on the tracing.

Fig. 5. 2 c. c. of 50 % bile injected in 20 seconds. The pulse became very slow and the pressure fell very rapidly to a dangerous depth. At the end of the injection the pressure rose rapidly to above the original level, only to fall again immediately to a fatal level.

Fig. 6. At the arrow the slow injection of 5 c. c. of undiluted bile began and was finished at the cross at the bottom of the curve in 3 minutes. The

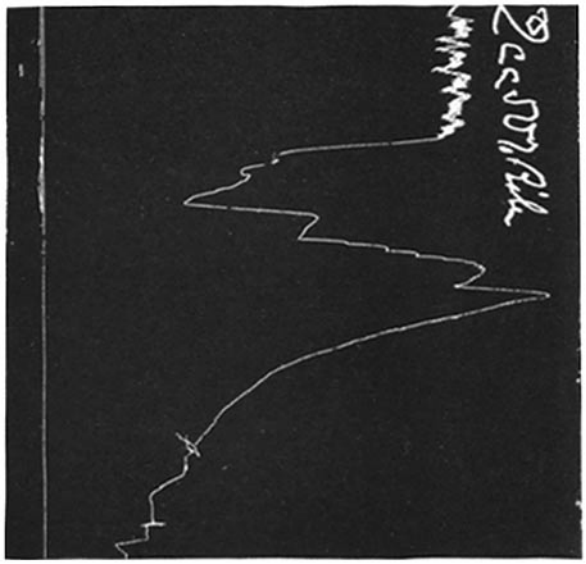

FIG. 5.

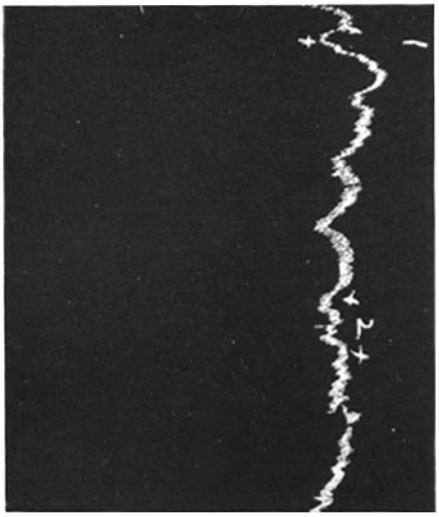

FIG. 2.

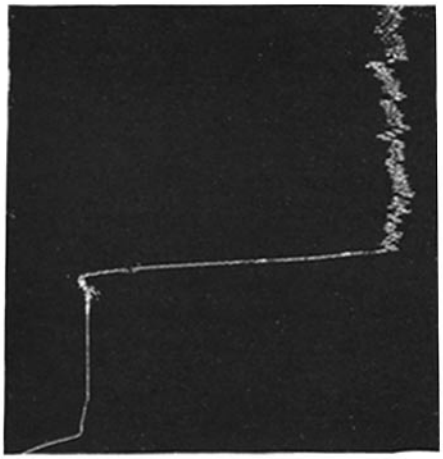

FIG. 1.

FIG. 4.

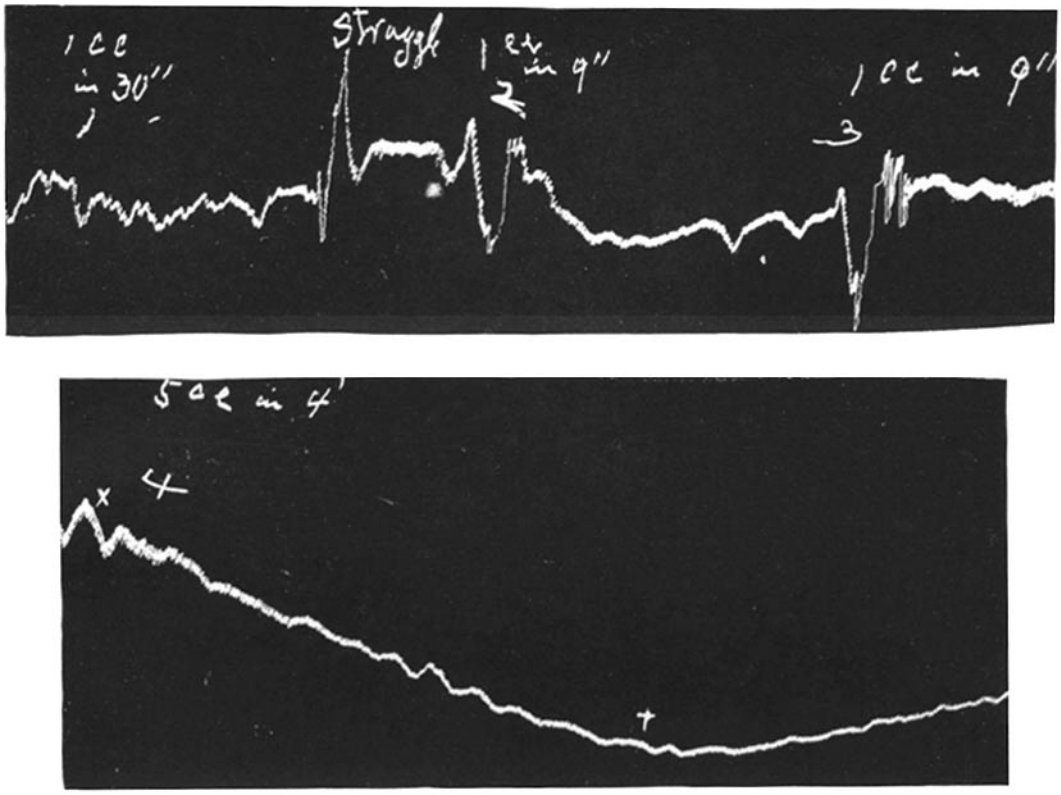

FIG. 3.

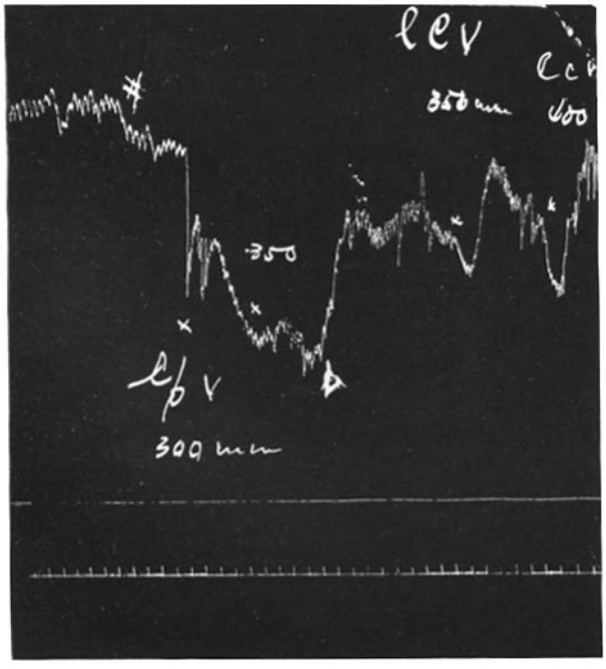

FIG. 8.

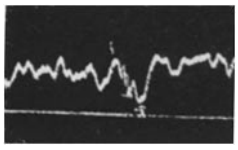

FIG. 7.

FIG. 6.

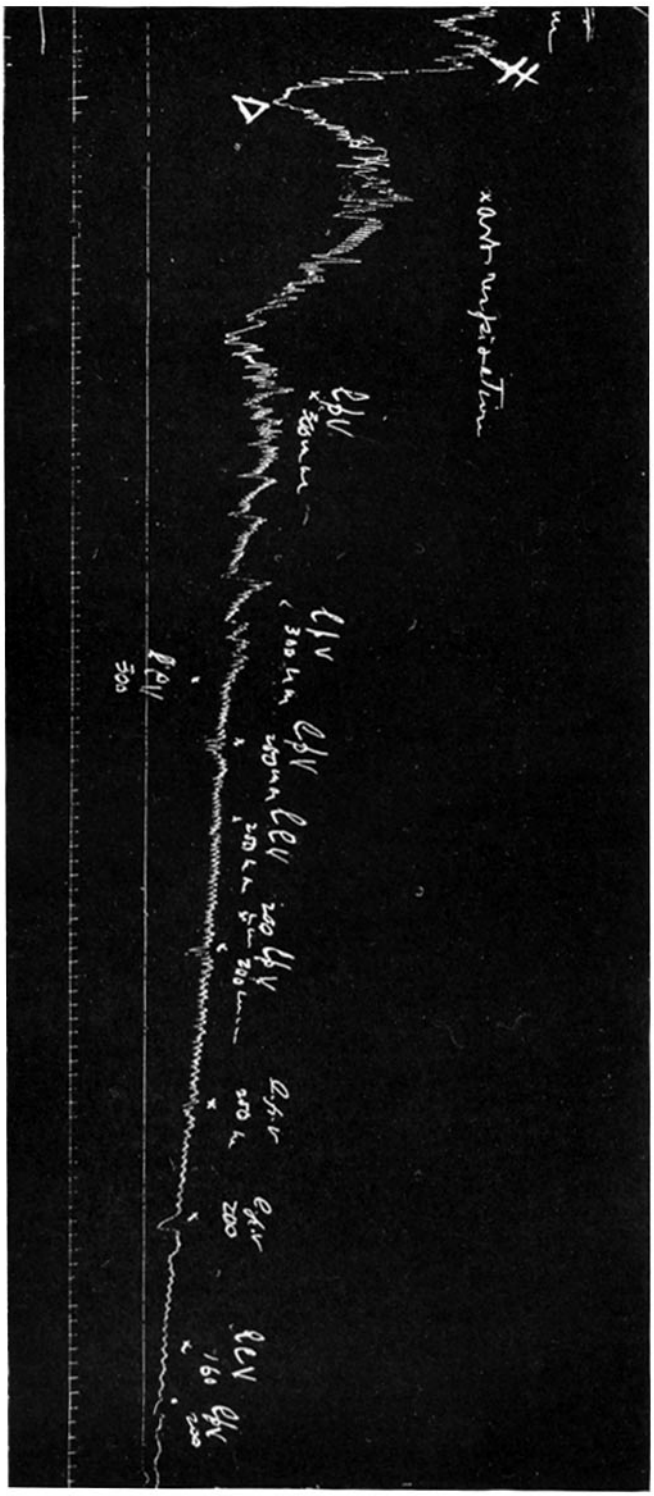

FIG. 9.

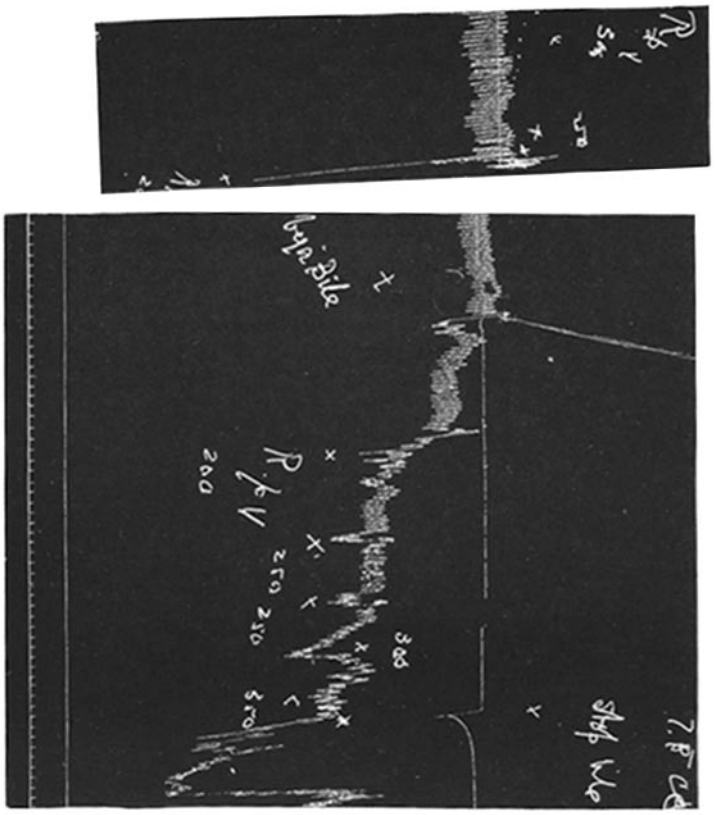

FIG. 10.

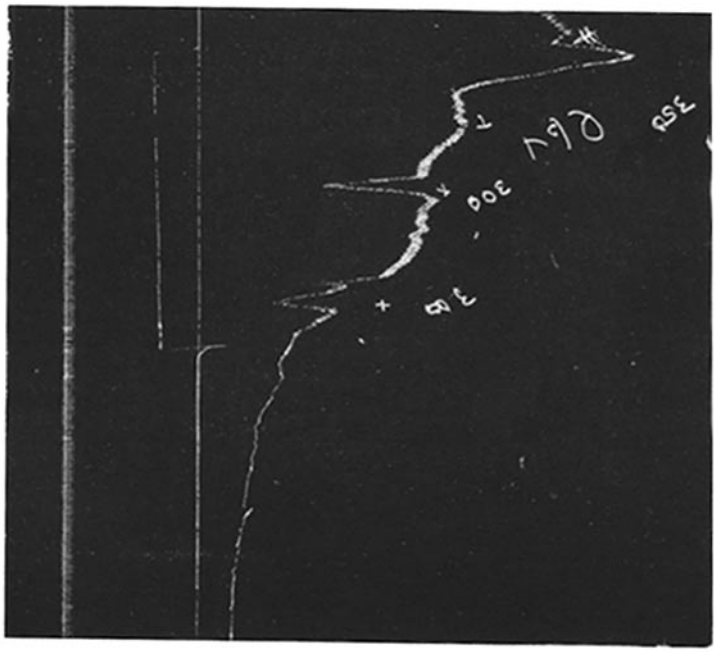

FIG. 11.

descent began after the waves disappeared, and the return began a little while after the injection was finished.

Fig. 7. 1 c. c. of a 2 % bile injected in 10 seconds. The fall hardly amounts to 12 millimeters of mercury.

Fig. 8. 1 c. c. of a 20 % solution of inspissated ox bile was injected in 1 minute, beginning at × and finishing at △. At + l. p. v. means that the left peripheral vagus was stimulated by an electric current from an induction when the secondary coil was 300 millimeters distant from the primary coil (Kronecker's induction coil with one Daniell cell). The heart stopped and the blood pressure dropped straight down; 350 mm. distance had no effect. l. c. v. means that the left central vagus was stimulated with 350 and 400 mm. distance at ×, and it caused reflexly a drop which was followed by a rise after discontinuance of stimulation. The middle line is the base line, the lowest line time in 4 seconds.

Fig. 9. 7.5 c. c. undiluted bile injected in 3 minutes. On the left-hand side vagus tested (see description of Fig. 8) before injecting the bile; 300 and 250 mm. distance no effect; 200 mm. causes immediate standstill and a drop of pressure in straight line (number beneath this line). While injecting the bile, 250 and 300 mm. became distinctly effective; a sudden fatal drop followed; artificial respiration ineffective. Line through blood-pressure line marks beginning and end of bile injection. Lower lines, base line, and time-marking in 4 seconds.

Fig. 10. 1 c. c. of a 20 % solution of inspissated bile injected in 20 seconds. × = beginning, △ = end of injection. Blood pressure fell to a fatal level and artificial respiration instituted; blood pressure rose at first, then dropped and continued to sink until 2 mm. above the base line death occurred. During the continued sinking, vagus was tested (see description of Fig. 8). There was still a distinct inhibitory effect when blood-pressure was hardly more than 5 mm., and the heart-beats were barely perceptible. See last mark l. v. p. 200. The two lines beneath blood-pressure curve are base line and time-marking line in 4 seconds.

Fig. 11. 6 c. c. undiluted bile injected in 6 minutes. Fatal fall of blood pressure. Beginning at ×, slight rise preceding the beginning of the fall; △ = end of injection. Vagus tested (see description of Fig. 8); marked inhibitory effect at 300 mm. distance, improved on preceding test which is not reproduced. Lines beneath pressure curve: base line, time-marking line, 4 seconds, slow revolving drum; the line between indicates the time of the injection of the bile.