STUDIES ON THE TOXICITY OF BILE.

II.

THE TOXIC EFFECTS OF BILE UPON THE CENTRAL NERVOUS SYSTEM AND THE ELIMINATION OF STRYCHNINE THROUGH THE BILE IN NEPHRECTOMIZED ANIMALS.

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The occurrence of severe nervous manifestations in the course of diseases in which icterus is a predominant symptom gave rise at an early period to experimental investigations of the effect of bile upon the animal organism. During the first part of the last century the methods employed in these investigations consisted mainly in intravenous injections of ox bile obtained from the gall bladder. As in most of the experiments the injections caused the death of the animal, it was ascribed to the toxic effect of the bile. During the forties of the last century, however, the view gained ground that death following intravenous bile injections was not due to the toxicity of the bile, but to impurities in the fluid used which give rise to the formation of capillary thrombi in vital nerve centres. Thus Bouisson reported that animals which received filtered bile survived the intravenous injections without manifesting any important nervous symptoms. This result could not have failed to exert a deciding influence upon contemporary opinion, and we need not therefore be surprised to find Henle [1] making the statement that the widespread view of the toxicity of bile was an unwarranted assumption. However, after the discovery by Strecker [2] of bile acids, numerous investigations were carried out with them, and any question of accidental causation of thrombosis could therefore no longer

[1] Henle, *Handbuch der rationellen Pathologie*, 1847.

[2] A. Strecker, *Habilitationsschrift*. Giessen, 1848.

enter seriously into the discussion of the results. The first extensive study of the toxicity of bile salts was made by von Dusch.[3] And among the subsequent investigators we find such well-known names as Frerichs, Leyden, Kühne, Traube, Hoppe-Seyler, Landois, and many others. The last investigation of this subject, so far at least as the effect upon the nervous system is concerned, was carried out some fourteen years ago under Kobert's direction by D. Rywosch.[4] It was a comprehensive, painstaking piece of work which has left its impression upon all later writings on the subject of the toxicity of bile.

The present status of the views entertained concerning the toxicity of bile may be described as follows: All agree that bile is a very toxic substance. It hæmolyzes red blood corpuscles and dissolves white blood corpuscles and other tissue cells; it retards and stops the heart-beat, lowers the blood pressure, and is capable of affecting the structure of nerve and muscle, etc. Regarding the effect upon the central nervous system, there is unanimity of opinion that bile produces depression; most of the investigators describe stupor and weakness of the animals as the predominant symptoms following the intravenous injection of bile salts in mammals, and coma and paralysis following their injection into the lymph-sacs of frogs. A few investigators have also observed occasionally the occurrence of convulsions. von Dusch [5] describes three experiments in which the animals died in convulsions a few minutes after receiving intravenous injections either of filtered bile or of sodium cholate. Röhrig [6] observed similar effects in two experiments in which 7 c.c. of a 5 per cent. solution of sodium cholate were injected into the jugular vein. Leyden [7] described a few experiments in which convulsions appeared after injection of bile salts into the jugular vein; in one case the convulsions appeared immediately after injection of one c.c. of a 10 per cent. solution of sodium glycocholate. In the vast literature on the studies of bile there are scattered records of a few more experiments in which convulsions occurred among the effects of the injection of bile. However, even Leyden, who more than any other author insisted upon the appearance of convulsions after intoxication with bile, states that after all it is a rare occurrence. We [8] have ourselves stated in a previous paper that in most experiments the convulsions set in soon after the intravenous injection of bile or bile salts, and have expressed the view that they were simply terminal manifestations due to heart failure produced by too rapid injection of the solutions. In a few exceptional cases convulsions are recorded to have

[3] von Dusch, *Habilitationsschrift.* Leipzig, 1854.
[4] D. Rywosch, *Inaugural Dissertation.* Dorpat, 1891.
[5] von Dusch, l. c.
[6] Röhrig, *Dissertation.* Würzburg, 1863.
[7] Leyden, *Beiträge zur Pathologie des Icterus.* Berlin, 1866.
[8] Meltzer and Salant, *Journal of Experimental Medicine,* 1905, vii, 280.

developed a day or two after subcutaneous or intraperitoneal injections. Since, however, in these instances the bile or bile salts tend to produce extensive local necrosis, the convulsions are not necessarily to be regarded as due directly to the bile; they may have been caused by absorption of toxic products from the necrotic areas, or the result of a terminal infection. In frogs the toxic symptoms appear so soon after the injection of bile or bile salts into the lymph-sacs as to be ascribed directly to bile intoxication. Leyden,[9] Loewit,[10] and Rywosch [11] tested bile salts upon frogs, and report that coma and paralysis are the only effects which they have observed. Rywosch indeed asserts that in all his experiments, in which mammals were also included, he never saw anything but a depressing effect. It was this last statement which caused the more recent writers on diseases of the liver to abandon the old theory of cholæmia as due to bile absorption in acute yellow atrophy of the liver or of any other form of *icterus gravis*. The symptoms are sometimes of a depressing character, as in coma, etc., and sometimes manifestations of increased excitability, as in convulsions or excitation mania. On account of the occurrence of the latter symptoms, Stadelman [12] and others insisted that bile absorption could not be the cause, and they argued that bile causes only coma and similar symptoms of a depressing nature, but never convulsions.[13]

To sum up briefly, it may be said that bile, according to the present almost universally accepted view, contains a toxic element which is capable of producing nervous depression, coma, and paralysis, but does not contain any element which can produce excitation and convulsions.

Our experiments have consisted in the injection of bile and bile salts into animals. We have employed chiefly rabbits' bile, although bile from the ox, dog, and guinea-pig was also used. In addition to this, experiments with glycocholate and taurocholate of sodium were made. The frog was chiefly employed for the injections, which were made into the dorsal or ventral lymph-sacs. There are many reasons for selecting frogs for the experi-

[9] Leyden, l. c.

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

[11] Rywosch, l. c.

[12] Stadelman, *Ueber Icterus*. Stuttgart, 1891.

[13] The recent observation by Biedl and Kraus [14] that subdural injections of bile cause convulsions can hardly be taken as a valid proof that bile contains a tetanizing element, since even minute doses of such comparatively innocent substances as methylene blue, which by intravenous injection are almost harmless to the animal organism, can produce convulsions when injected subdurally or intracerebrally.[15]

[14] Biedl and Kraus, *Centrabl. f. klin. Med.*, 1898, p. 1185.

[15] Bruno, *Deutsche med. Wochensch.*, 1899, xxv, 369.

ments, for, from what we have stated above, it is evident that mammals are not very suitable for them. When in mammals the injections are made intravenously, the rapid reactions which often follow are due to the effect of the bile upon the heart, and not to a primary effect upon the nervous system. When, on the other hand, the injections are made into the peritoneum, etc., the reactions sometimes set in so late as again to permit of doubt as to whether they are or are not primary toxic effects. The case with frogs is different. The reactions which follow the injections of bile into the lymph-sacs set in early enough to be due directly to the bile intoxication; nor can there be any suspicion that the nervous manifestations are secondary to some disturbance in the circulatory or respiratory systems.

As the main title of this paper indicates, the chief part of this research was devoted to a study of the toxic effects of bile upon the central nervous system. This, however, was not the primary consideration which started us on this long investigation; for, indeed, we were in quest of a solution to a problem which presented itself to us in the course of our previous researches. Before the latter could be attacked, we became confronted with the question of the normal toxicity of bile, in the study of which new thread we arrived at results which shed light on this new problem, while, at the same time, it assisted in elucidating the original problem. And herein will be found the chief reason for using rabbits' bile upon frogs. In presenting our results it would be natural to follow the tortuous path by which we gradually arrived at them. We shall therefore begin with the statement of the original problem which started us on this research.

It is an established fact that strychnine when introduced into the body by any path leaves it entirely through the kidneys. We[16] have, however, found that after double nephrectomy, two or three times the fatal dose of strychnine can be injected without causing intoxication, provided each single quantity injected is less than the minimum toxic dose, and the intervals between the injections are not too short; that is, it appeared that after nephrectomy there is no cumulative effect of the strychnine in the

[16] Meltzer and Salant, *Journal of Experimental Medicine*, 1901–1902, iv, 107.

blood. One of the possible explanations for this remarkable condition lies in the assumption that after nephrectomy the process of elimination is assumed by another organ. The gastro-intestinal canal suggested itself to us as this channel,[17] but we failed to detect strychnine in it. Later, [18] it was found that the methods at hand for detecting traces of strychnine make it difficult to discover small quantities of it in the intestinal canal. Hence, in the further pursuance of the question of vicarious elimination, we turned our attention to the bile through which, it is claimed, poisons are normally eliminated from the body. Our problem therefore was to determine whether, after nephrectomy, strychnine is eliminated through the bile. As our previous experiments were made on rabbits, it was necessary therefore to make the new ones on the same animal. Hence our reason for using rabbits' bile in this investigation. Furthermore, as the chemical method for separating strychnine from bile is very tedious, and would become quite unmanageable if, as in our plan, a large number of experiments were to be performed, we resorted to the biological test—i. e., the injection of bile supposed to contain strychnine into frogs in order to produce the usual well-known strychnine convulsions.

In attempting to solve this problem several series of experiments were made. In one series the rabbits which were nephrectomized had their common bile duct ligated. After recovery from the operation the animals were given several subminimum doses of strychnine. The animals were studied first with reference to the amount of strychnine they could stand without suffering a convulsion, and second, after the death, in that the bile from the gall bladder and the dilated duct was injected into frogs. In another series of experiments a cannula was tied in the common duct of the nephrectomized rabbits, and, after injecting subcutaneously subminimum doses of strychnine, the collected bile was tested on frogs. The following is a brief *résumé* of these experiments.

Series I.—*Both kidneys removed, the common bile duct ligated,*

[17] Salant, *American Medicine*, 1902, iv, 293.

[18] Salant, *Journal of Medical Research*, 1904, xii, 41.

and, subsequently, subminimum doses of strychnine injected at varying intervals.

This series included seven rabbits. Four showed no signs of a cumulative effect, although the sum total of the several doses of strychnine in each animal exceeded by far the minimum fatal dose (about 0.6 milligramme per kilo). One animal lived 74 hours and received during that time nearly 3 milligrammes per kilo without showing either convulsions or hyperæsthesia. It died under the symptoms of exhaustion.

The behavior of these four animals was identical with that of nephrectomized rabbits in which the common duct was not ligated.

Of the remaining animals, one received within twelve hours about 0.9 milligramme per kilo of strychnine without showing any effects. Three hours later, however, when it was again given about 0.2 milligramme per kilo, the animal had a tetanus and died in a few minutes. The course in this experiment did not differ materially from that which sometimes occurred in our previous experiments on nephrectomized animals without ligation of the duct, which received strychnine in refractive doses.

The remaining two animals, however, died in violent convulsions two hours after receiving only 0.3 milligramme of strychnine per kilo, a dose which is, as a rule, non-toxic even for normal animals.

These observations, then, did not permit of any positive conclusions being drawn one way or the other. In the majority of the animals the closing of this additional eliminating path did not increase in the slightest the cumulative effect of the poison within the blood, a fact which would speak against the elimination of the strychnine through the bile. On the other hand, in two experiments, ligation of the bile duct did produce a rather striking aggravating effect upon the injection of a single subminimum dose of strychnine. Furthermore, we had to take into consideration that the absence of strychnine symptoms in the majority of these animals could not be taken as an absolute proof of the absence of strychnine in the bile, because it was possible that the refractive doses of strychnine were eliminated from the blood by, and accumulated largely in, the bile within the gall bladder, but through the abnormal state of the bladder under these conditions the strychnine was prevented from being re-absorbed into the circulation, which would account for the absence of convulsions in most of the experiments.

In the latter case we would expect that the bile of these

animals when injected into frogs would produce symptoms of strychnine poisoning. This view was tested. Bile was taken from the gall bladder and the dilated bile duct from four rabbits of this series. The quantities were of course small and only a few experiments could therefore be made. The few results were as follows:

One c.c. of the bile injected into a medium-sized frog caused coma in one hour; and ½ c.c. caused in a small frog complete coma and paralysis half an hour after injection. As to the remainder, it can be briefly stated that none of the frogs which received smaller or larger doses of the bile showed convulsions or hyperæsthesia.

These few observations did not indicate that the bile contained strychnine, at least not in such quantity as to set up characteristic symptoms in frogs.

Regarding the preceding experiments it may be said that they were capable of providing positive proof only if all the animals had manifested strychnine poisoning, or in other words if the bile of the animals had contained strychnine we should have had evidence that after nephrectomy strychnine is eliminated in this secretion. However, the failure of these experiments to bring forward this proof can not be interpreted as disproof, namely, that the biliary organs do not assume the function of elimination of strychnine after nephrectomy; for, just as we have assumed that after removal of the kidneys another organ, the liver, takes over the function of elimination, we may now assume that after nephrectomy and ligation of the common duct still another organ assumes the work of elimination of strychnine; whence the failure of the above experiments to show that the bile contained strychnine. We expected to obtain more satisfactory evidence regarding this assumption from the following set of experiments.

Series II.—*Rabbits were nephrectomized and a cannula placed in the bile duct; strychnine in subminimum doses injected, and the bile collected and introduced into frogs.*

We made six such experiments. In five animals some bile was collected before the strychnine injections were given. This bile was also injected into frogs which served as controls. All the frogs receiving bile from the ante-

strychnine period responded with coma and all but one became paralyzed and died. None of the frogs showed tetanus, rigidity, or convulsions, and all but one showed even no hyperæsthesia. One showed marked hyperæsthesia, but soon also passed into coma and paralysis.

The frogs which received the bile from the strychnine period presented a different picture. The bile from one rabbit which received within six hours 0.7 milligramme of strychnine nitrate caused in three frogs tetanus and death in twenty minutes. The bile from another rabbit which received 2 milligrammes of strychnine in forty-eight hours produced tetanus in two frogs, marked hyperæsthesia in one, and had no effect in another; in the last case a dose of bile as large as three c.c. was given. Two rabbits were given about 0.3 milligramme of strychnine; the bile of one produced tetanus in one, marked hyperæsthesia in another, and coma and paralysis in a third frog. The bile of the other produced late rigidity and convulsions in one and coma in another frog. Of the remainin ; two rabbits, one received 0.5 milligramme of strychnine in a single dose; the bile of this animal caused in one frog hyperæsthesia which terminated in coma, and in another it produced only coma. The same effect resulted from the bile of the last rabbit, which received a single dose of strychnine (the amount is not noted in the protocols).

The bile (strychnine period) from the rabbits of this series produced distinct strychnine symptoms (hyperæsthesia, etc.) in nearly all the frogs, while distinct tetanic attacks were produced by the bile of rabbits which received at least toxic doses. Comparing these results with those obtained with the bile from the ante-strychnine period of the same series, the conclusion seems to be justified that in these animals some of the strychnine at least was eliminated through the bile.

New questions now arose. In the first place, the question as to whether the presence of strychnine in the bile of this series of animals represents a vicarious process only, and whether in normal animals which receive strychnine the bile does not also, as a rule, eliminate part of the poison; and in the second, whether the bile of nephrectomized animals, not receiving strychnine, does not contain a tetanizing element of metabolic origin, which may have caused the hyperæsthesia and convulsions described in the last series of experiments. These questions were tested by short series of experiments.

Series III.—*Bile collected from a cannula in the common duct of normal rabbits before and after the injection of strychnine and tested on frogs.*

Only three such experiments were made. One animal received 0.9 milligramme of strychnine. Two c.c. of the ante-strychnine bile of this animal gave coma which developed gradually within one hour. Two c.c. of the bile of the strychnine period gave marked hyperæsthesia, and the addition of two c.c. more of the bile brought out immediately a violent tetanus which passed over rapidly into coma and paralysis.

In the second experiment the rabbit received one milligramme of strychnine. Two c.c. of the ante-strychnine bile gave coma as before, whereas the same amount of bile of the strychnine period gave pronounced hyperæsthesia, which after an hour passed over suddenly into coma (a very brief period of convulsions might have been overlooked). In both the experiments the urine collected after the administration of strychnine was also tested; it was almost without effect.

The third animal received 1.5 milligrammes of strychnine. Two c.c. of the bile (before and after strychnine injection) produced no effect. Three c.c of the bile of the strychnine period produced coma, while three c.c. of the bile of the ante-strychnine period produced rigidity of the frog. (The urine of this animal caused increased reflexes in frogs.)

Hence we have among this series two experiments in which the bile from non-nephrectomized rabbits, after injection of strychnine, produced marked hyperæsthesia and even a convulsion, while in the third experiment the normal bile of the rabbit (before receiving strychnine) produced symptoms distinctly similar to those of strychnine poisoning.

Series IV.—*Bile collected from the common duct of nephrectomized rabbits and tested on frogs.*

We had five such experiments. In one even three c.c. of the bile produced no effect upon the frogs, while three and a half and five c.c. brought on prolonged tetanic attacks. In another experiment three c.c. had no effect, and five c.c. produced hyperæsthesia, but neither convulsions nor coma. In a third experiment five c.c. brought on hyperæsthesia and stupor. Of the bile of the fourth experiment three c.c. had no effect, but five c.c. brought on coma and paralysis. Finally, in the fifth experiment two c.c. of the bile produced coma and paralysis.

In this series, with entire absence of strychnine, marked hyperæsthesia was a frequent phenomenon, and distinct tetanic attacks occurred. On the other hand, coma and paralysis were seen only in two instances and in one instance only after injecting five c.c. of the bile.

A remarkable fact of this series is that in nearly all of the experiments as large doses of bile as three c.c. produced no effect at all.

The control bile of all the previous experiments (except one) produced with two c.c., and sometimes even with one c.c., distinct coma and paralysis. The ante-strychnine bile in the second series was also obtained from nephrectomized rabbits and is therefore comparable with the bile of the last series in every respect except one, that it was obtained at the very beginning of the flow of bile from the cannula. This latter fact might indeed be an important feature and perhaps explain the absence of hyperæsthetic and convulsive effects from the bile of the ante-strychnine period of the second series. We may, perhaps, assume that the hypothetical tetanic element, which normally is eliminated through the urine and after nephrectomy finds its way out of the body through the bile, appears in the bile in effective doses only a time after the removal of the kidneys —that is, that the vicariation develops only gradually. The bile of the fourth series was collected during a long period, sometimes extending over two days and longer, after the nephrectomy, whereas the bile of the ante-strychnine period of the second series was collected only an hour or two immediately following the nephrectomy. Hence, the absence of a tetanic element in the one and its frequent occurrence in the other bile.

However this explanation may account satisfactorily for the production of hyperæsthesia and convulsions in the experiments with the bile of the fourth series, it does not explain the remarkable reduction in toxicity. What could the factor be that caused the surprising reductions in the elements producing coma and paralysis in the bile of the fourth series so that even such quantities as three c.c. had no effect?

Could the presence of a tetanic element in the bile, be it strychnine or some catabolic product, influence the activity of the elements producing coma and paralysis normally present in the bile so as to neutralize to some degree the depressing effect?

Before this question could be properly disposed of, a more fundamental proposition confronted us and had to be studied, namely, as to what the actual effect of the bile collected for a longer period from the common duct of a normal animal is. In

the preceding series we had not yet dealt with this question. The control bile of the third series, which was a normal bile, was collected only for a short period and soon after the introduction of the cannula into the bile duct, in which instances the possible after-effect of the anæsthesia had to come into consideration. But even in this short series the results were not uniform. The control bile of the three experiments produced coma and paralysis in two and rigidity in one animal.

In reviewing the literature of this subject we learned that the opinion now generally held is that bile produces coma and paralysis. It would seem as if only three investigators experimented upon frogs. Leyden made injections in three, and Rywosch in four frogs, while Loewit, who probably made many injections, states his general conclusions only. The three investigators employed chiefly biliary salts, or when bile was used it was ox bile obtained from the gall bladder. It has been generally assumed that bile obtained from the gall bladder is more poisonous than that obtained from the common duct. It is therefore evident that there has been as yet no information whatsoever secured as to the behavior of bile collected from the common duct of normal rabbits. We have studied this subject by means of a large number of experiments, and will now proceed to report briefly the results obtained from them and from others made with stagnant bile from the gall bladder and the dilated, ligated ducts of otherwise normal rabbits.

Series V.—*Bile of normal rabbits.* a. *Bile from the gall bladder or from the dilated, ligated duct.* b. *Bile collected from a cannula in the common duct.*

a. We made only a moderate number of these experiments. The results were as follows. As small quantities as one-half c.c. produced no immediate effect, but most of the animals were found dead the next day. One c.c. produced, as a rule, coma and paralysis an hour or two after injection. In two instances even one and a half c.c. produced only a moderate effect which set in late. (It seemed that injections into the ventral brought out the effect more promptly than into the dorsal lymph-sac. Possibly the abdominal pressure in the normal posture acts like massage and hastens absorption.) In no case was any increase of reflexes, or convulsions, or rigidity observed.

b. As a rule large quantities of bile were collected in this manner, sometimes more than 100 c.c., and thus experiments could be performed upon many

frogs with the bile obtained from one animal. The data which we give were gathered from experiments made with bile of nine different rabbits. In many of the numerous experiments the employment of the normal bile was a part in some experiment in which the normal bile served as a control for comparison with the effect of bile of some other kind.

The chief result of this series of experiments is the establishment of the fact that the bile of some rabbits can produce distinct hyperæsthesia and tetanic attacks. Whether the bile is moderately or strongly toxic, and whether the predominating toxic effect is depressing, as in coma and paralysis, or exciting, as in hyperæsthesia and convulsions, seemed to depend greatly upon the individuality of the animals. Our experience with these animals and with other experiments in the course of this research, led us to feel justified in dividing rabbits into three groups according to their individualities as above indicated.

The bile of one of the groups caused coma and paralysis essentially. Two c.c. would, as a rule, produce these symptoms within a few hours, and the larger the quantity injected the sooner would they set in and the more complete would they be. Quantities less than this but above one c.c. produced during the eight or ten hours of observation scarcely any perceptible effect, but on the next day or the third day the animals would be found dead. One c.c. of bile was almost without action. The bile of these animals produced neither hyperæsthesia nor convulsions no matter what the amount given. We wish to emphasize that the bile of this group of animals produces coma and paralysis but never hyperæsthesia and convulsions.

In a second group of animals bile in quantities of two c.c. or even two and a half c.c. produced no effect whatever. Three c.c. of bile caused sooner or later more or less marked hyperæsthesia, from which the majority of the frogs recovered. Doses of five c.c. produced symptoms of coma and paralysis more or less distinct. As the doses were increased, the symptoms intensified and appeared the sooner. These animals all succumbed. The main characteristic of this bile is that small doses produce hyperæsthesia and larger ones coma and paralysis. In exceptional cases coma would be preceded by a convulsive attack of

short duration and easily overlooked. On this account we may have missed certain of their manifestations.

The bile of the third group in doses of from two to three c.c. produced at most no effect. Three and a half c.c. or more produced marked hyperæsthesia passing into clonic or tonic convulsions. Sometimes a violent tetanus would set in two or three hours after injection of the bile, and at other times the tetanic effect appeared the next day, or only after prolonged handling of the frogs; it tended to be of a pronounced tonic character, the animal remaining uninterruptedly rigid for two minutes or longer. Again the tetanic effect appeared only after such large doses as six, seven, or more c.c. Some frogs survive mild tetanic attacks and return to a good condition. Those which had violent or prolonged tetanic attacks did not, as a rule, recover, but behaved as if poisoned with a toxic or fatal dose of strychnine. None of the frogs which received bile of this group of animals ever showed coma and paralysis.

The bile of the first or coma-producing group was toxic in small doses; the bile of the third or convulsive group exerted a toxic effect only in larger doses; the bile of the intermediate group, producing hyperæsthesia in smaller, and coma in larger doses, possessed only moderate toxicity.

In no instance did we encounter a bile which in small doses caused coma and in larger ones convulsions or hyperæsthesia.

These experiments established beyond doubt that the bile of some rabbits contains a pronounced tetanic element. In certain specimens of bile the tetanic constituent was the only noticeable toxic agent, in others the depressing toxic element existed in moderate amount, the tetanic effect manifesting itself only by hyperæsthesia and in those doses which did not exert a depressing effect. In one kind of bile only could no evidence of the presence of a tetanic element be discovered, namely, in that which in small doses exerted a strong depressive action leading to coma and paralysis.

The question which now arose was this: Does the bile which produces only coma and paralysis actually contain no tetanic element? From the behavior of the bile of the intermediate

group we had reason to believe that the element which produces coma and paralysis, when present in sufficient strength, is capable of suppressing the manifestations of the tetanic constituent. It is therefore conceivable that the very depressing bile of the first group contains a tetanic body, the manifestation of the action of which is rendered impossible because of the fact of the suppressive action of the depressing element.

In following out this possibility we succeeded in demonstrating the presence of a tetanic element in the bile of all the rabbits studied.

We had observed that a medium dose of bile of the intermediate group was capable of bringing out hyperæsthesia although the bile surely contained a certain amount of the depressing element. The reason for this result apparently rested on the fact that in this dose of bile, which was subminimum for the depressing effect, the mutual relations between the tetanic and the depressing substances were in favor of the tetanic agent. We now thought that by artificially supporting by strychnine in ineffective doses the tetanic element, when present in the bile in subminimum doses, it might also be possible to develop the hyperæsthesia and perhaps even the convulsions.

In a previous part of this paper we were led to consider whether strychnine or a tetanic substance of catabolic origin might not be capable to some extent of overcoming or neutralizing the depressing effect of the coma and paralytic elements. Stimulated by these questions, we carried out several series of experiments in which the effects of bile supported by additional injections of strychnine were studied.

Series VI.—*Bile and strychnine injected into frogs.*

In a few experiments strychnine was mixed with the bile in certain proportions and kept for some time before injection. In most of the experiments, however, bile and strychnine were injected consecutively in different parts. The doses of strychnine were known from many previous experiments to produce either no effect or a very slight one; besides which facts, in many of the tests the dose employed was carefully controlled.

At the outset it will be well to state that in all cases the addi-

tion of a subminimum dose of strychnine brought out perceptible effects which were not seen in the control experiments with strychnine or bile alone.

Group I.—Bile from nephrectomized animals and strychnine were mixed in proportion of 1 : 10,000, kept for 24 hours, and then injected into frogs.

Small frog A, control; injected into d. l. s.* 0.01 mgr. strychnine nitrate at 3.17 P.M. Observed until 5 P.M.; no increase of reflexes. Next day normal.

Frog A′ size of control; injected into d. l. s. 0.1 of a mixture of 0.1 bile + 0.01 mgr. strychnine at 3.22 P.M.; 4.07 reflexes distinctly increased; 4.15 violent tetanus. Next day again normal.

Small frog B, control; injected into d. l. s. 0.02 mgr. strychnine nitrate at 11.23 A. M. At 12.15 P.M. no increase of reflexes; 12.55 reflexes slightly increased; 1.10 on handling, a mild tetanic attack which soon passed over.

Frog B′ size of control; injected into d. l. s. 0.2 of a bile mixture of 0.2 bile + 0.02 mgr. strychnine nitrate at 11.28; 12.04 violent tetanus.

Large frog C, control; injected into d. l. s. 0.05 mgr. strychnine nitrate at 11.52 A.M. 12.53 P.M. no increase of reflexes; 1.15 reflexes increased; 2.10 reflexes markedly increased; 2.22, violent tetanus.

Frog C′ size of control; injected into d. l. s. 0.5 of a bile mixture of 0.5 bile + 0.05 mgr. strychnine nitrate at 12 M. 12.40 P.M. marked increase of reflexes; 12.53 violent tetanus.

The presence of a tetanic element in the bile in this group of experiments is quite evident. The doses of bile employed never alone produced hyperæsthesia, to say nothing of tetanus. Nevertheless one sees in the three experiments a marked increase in the efficiency of the strychnine produced by the addition of the bile.

Group II. Frog A, control; injection of 0.01 mgr. strychnine nitrate. No effect. Frog A′ injected 0.01 mgr. strychnine + 1.5 salt solution (0.85 %). After 3½ hours very slight temporary increase of reflexes (?). Frog A″, 0.01 strychnine + 3 c.c. salt solution. After 3½ hours slight temporary increase of reflexes (?). Frog A‴, 0.01 mgr. strychnine + 5 c.c. salt solution; same as the others. All practically normal.

Frog B, injected 1.5 c.c. bile from common duct of a normal rabbit at 10.30 A.M. Condition normal; next day the same. Frog B′, injected 1.5 c.c. same bile as above and immediately after injected 0.01 mgr. strychnine nitrate at 10.30 A.M. At 2 P.M. reflexes markedly increased, and at 2.10 on handling, tonic rigidity. At 6.30 reflexes still increased. Next day frog perfectly normal.

Frog C, injected 3 c.c. of the above bile at 10.30 A.M.; at 2 P.M. increased reflexes; soon coma; at 6 P.M. dead.

* The abbreviation d. l. s. will be employed to designate the dorsal lymph-sac.

Frog C′, injected 3 c.c. bile and immediately after 0.01 mgr. strychnine nitrate. At 2 P.M. increased reflexes; no coma; at 6.30 P.M. reflexes markedly increased and no coma. Next day animal perfectly normal.

Frog D, injected 5 c.c. of normal bile; at no time increased reflexes; coma and paralysis soon appeared, and later in the day the animal was found dead.

Frog D′, injected 5 c.c. of normal bile at 11 A.M.; animal soon drooped; injected 0.01 mgr. strychnine. One hour after the injection of strychnine a tetanus broke out; at 6 P.M. animal still tetanic. Recovered next day and remained perfectly normal.

This group of experiments is very instructive. Doses of strychnine, the ineffectiveness of which was established in many controls, brought out distinct specific symptoms when given with bile, and with the increase of the dose of bile, although the dose of strychnine remained the same, the strychnine symptoms increased in intensity, so as to bring on a distinct tetanic state, lasting many hours. This result can only mean that normal bile contains a tetanic element or a substance which in its effect is similar to that of strychnine.

Moreover, this experiment demonstrates a still more important fact. In C and D doses of three and five c.c. of normal bile produced coma, paralysis, and death, whereas in C′ and D′ by the addition of a subminimum dose of strychnine not only was a tetanus brought out, but coma and paralysis were also prevented and the animals survived the fatal dose of bile.

It will be recalled that the bile from the common ducts of certain rabbits produced, in comparatively large doses, hyperæsthesia and convulsions. This result means that in these doses the tetanic element is present in at least minimum toxic doses.

The bile of some other rabbits causes in comparatively small doses coma and paralysis. We have demonstrated that even this bile contains the tetanic element. That this element fails to manifest its presence when injected without the addition of strychnine is probably due to the overpowering influence of the depressing element of this bile. Some of the cited experiments show, and it will be shown more fully later, that the depressive substance in bile, when present in effective strength, is capable completely of effacing the effects of a tetanic element, be it strychnine or a substance of metabolic origin.

It will be recalled also that the bile of some rabbits could be injected into frogs even in quantities of three or four c.c. without toxic effect of any kind. The explanation of this fact may be that in these specimens none of the toxic agents was present in such strength in the doses mentioned as to have a toxic influence. We have, however, reason to assume that in at least some instances the tetanic elements were strong enough in the doses employed to produce hyperæsthesia and convulsions. The harmlessness of this bile may, however, have been due to the simultaneous presence of the depressing element in quantities sufficient to neutralize the tetanic effects. It has been shown above that in some cases the dose of the bile alone would produce coma, but when a subminimum dose of strychnine was added to it the effect might be a tetanus. Hence the tetanic element of the bile plus the subminimum dose of strychnine were capable of antagonizing to such a degree a fatal dose of the depressing element as to produce a toxic effect in the opposite direction. We can under these circumstances easily conceive that were the strength of the tetanic element in the bile slightly less, the result would be a simple neutralization, and neither coma and paralysis nor tetanus be developed but the frogs would have appeared simply normal.

These neutralizations might occur in many ways. There might be, for instance, a certain kind of bile which in a certain dose contains exactly a minimum dose of the depressing element; the result would be coma and paralysis were it not for the simultaneous presence of the antagonistic tetanic element in a strength weak but sufficient to neutralize a fraction of the depressing element, thus reducing it to an ineffective strength. Or the tetanic element might be present in an exactly minimum strength and the depressing element in a fraction of a miminum dose. Neutralizations might perhaps also occur when both antagonistic elements are present in minimum doses or above them, provided they are present in such proportions as to be exactly sufficient to neutralize one another. As a rule, however, as will be seen later, such mixtures as the last do not lead to neutralization.

It should be stated that the cases in which the paralyzing effect of three and five c.c. of normal bile was completely overcome and turned into the opposite effect by the addition of strychnine, were not of frequent occurrence. We met them mainly in bile in which the depressing effect came out only when administered in still larger doses. As a rule, when moderate doses of bile were sufficient to produce complete paralysis and coma, the addition of strychnine was of no avail or had rather a contrary effect, as we shall see later. In cases, for instance, where the bile produced complete coma and paralysis with a dose of two c.c., we never succeeded in overcoming completely the effect by the addition of strychnine, although even in these instances some degree of neutralization was at times noticed. The following experiment illustrates this effect:

Group III.—Bile from the dilated common duct of a rabbit ligated three days before.

Frog A, injected 0.6 c.c. bile; complete coma and paralysis in one hour.

Frog A', injected 0.6 c.c. bile + 0.005 mgr. strychnine nitrate; some coma, increased reflexes, condition decidedly better than A.

Frog B, injected 1 c.c. bile; after one hour coma and paralysis, but alive.

Frog B', injected 1 c.c. bile + 0.005 mgr. strychnine nitrate; coma and paralysis very soon; dead at the end of an hour.

Here in A' the addition of a very small dose of strychnine was apparently instrumental in reducing the toxic effect of the depressing bile. (The effect in B' will be discussed later.)

The last two series of experiments have brought out two new important facts: 1. That rabbit's bile surely contains a tetanic element. 2. That the depressing and the tetanic elements within the bile are antagonistic to one another and are capable at least to a degree of mutual neutralization. Regarding the mutual relations of the antagonistic factors as they actually exist within the bile we have so far learned the following things:

In the bile of the gall bladder and the ligated common duct the depressing element is apparently the chief factor, the bile producing only coma and paralysis; it is toxic in comparatively small doses. The presence of the tetanic element can be detected only by the addition of an ineffective dose of strychnine and then

not in a very pronounced manner. Perfect neutralization is impossible.

The bile obtained from a biliary fistula of the common duct in general is far less toxic than that from the gall bladder. Rabbits show individual variations. The bile in some is already toxic in the comparatively moderate doses of two c.c. and less. The depressing element is predominant, the effects produced being coma and paralysis. This bile it is impossible to neutralize by the addition of strychnine. However, the doses which are not yet toxic permit without difficulty the bringing out of the tetanic effects by the addition of an otherwise ineffective dose of strychnine.

In still other animals the depressing element seems to be absent, at least it was so in doses up to ten c.c. This bile alone will produce a tetanus readily but only when given in the large doses of five c.c. or more. The addition of an ineffective dose of strychnine will bring out a tetanus with a somewhat smaller dose, but not with doses smaller than two c.c. In this bile, apparently, the tetanic element is also present in small quantities.

Finally, the bile of some rabbits produces hyperæsthesia in doses of two or three c.c. and coma and paralysis in larger doses. The addition of strychnine to comparatively small doses of this bile (one to three c.c.) will produce a tetanus. The addition of strychnine will bring out a tetanus also with doses of this bile just sufficient to bring out (without strychnine) coma and paralysis. When the dose of bile is distinctly above this minimum, coma and paralysis will be the result even if strychnine is added. Both elements are present and struggling against each other. In larger doses the depressing element is the victor, in smaller doses the tetanic element is capable of manifesting its presence, but produces only moderate symptoms. In doses near the border line the addition of strychnine is capable of turning the balance in favor of the tetanic element.

These various observations seem to demonstrate the following:

1. That for the depressing element there is a definitely effective minimum which, when present in a dose of any bile, overcomes

nearly completely the antagonistic tetanic element, no matter in what strength the latter is present.

2. That bordering on this effective minimum there is another minimum strength of the depressing element which, when present alone, or nearly so, can produce coma and paralysis; but this effect can be overcome by the antagonistic tetanic element when simultaneously present in the bile in a strength sufficiently above its minimum.

3. That this last minimum of the depressing element or a strength weaker but near to it, when mixed with a minimum effective dose of the tetanic element might lead through mutual neutralizations to normal effects.

4. That the tetanic element when alone is efficient in a much smaller strength than the strength which is required for the depressing element to be efficient; in other words: the effective minimum for the depressing element lies higher than that for the tetanic element.

With these relations of the antagonistic elements in mind, we can easily understand why some bile causes only depression, other bile only hyperæsthesia and convulsions, and still other bile produces hyperæsthesia in smaller and coma and paralysis in larger doses.

Remembering these considerations it may be further conceived that bile which would cause hyperæsthesia or even tetanus in small doses, might produce normal conditions with larger doses, and coma and paralysis with still larger doses. Such apparently paradoxical conditions would occur when both antagonistic elements were present in the bile simply in relatively minute strengths. The gradual increase of the dose of the bile would first bring the tetanic element up to its effective minimum while the depressing element would still be far below its effective strength, and the result would be hyperæsthesia or convulsions. A further increase in the dose of the bile would bring the depressing element up to a strength approaching its effective minimum, or just sufficient to neutralize the tetanic element even in its present strength; the result would be an apparently normal condition. Finally, a further increase of the dose of

bile would bring the depressing element up to the effective strength which is capable of completely overcoming the tetanic element in any strength; hence the coma and paralysis.

These paradoxical effects have actually been present in a few instances, especially in the experiments with additions of subminimum doses of strychnine. The following protocols are illustrations.

Group IV. Frog 1, injected 1.5 c.c. of bile from nephrectomized animals at 11.20 A.M. 2 P.M., frog normal. 6 P.M., rigidity of legs on handling; next day normal.

Frog 2, injected 3 c.c. of same bile at 11.10 A.M. 2 P.M., normal. 2.30 P.M., somewhat "stupid," otherwise normal, no increase of reflexes; next day normal.

Frog 3, injected 5 c.c. of same bile at 11 A.M. 2 P.M., coma and paralysis. 6.30 P.M., dead.

In this experiment Frog 2 had no rigidity or increased reflexes and was in better shape than Frog 1, while Frog 3 had coma and paralysis.

Group V. Frog 1, injected 1.5 c.c. bile from nephrectomized rabbit and immediately after injected 0.01 mgr. strychnine at 11.20 A.M. 2 P.M., violent tetanus, passed into coma. 6.30 P.M., found dead.

Frog 2, injected 3 c.c. of same bile, then 0.01 mgr. strychnine at 11.30 A.M. 2 P.M., increased reflexes. 6.30 P.M., increased reflexes, somewhat comatose (?). Next day normal.

Frog 3, injected 5 c.c. of same bile, then 0.01 mgr. strychnine at 11 A.M. 2 P.M., coma and paralysis. 6 P.M., dead.

Frog 2 with the larger dose of bile had neither tetanus nor coma and survived, while Frog 1 with the smaller dose showed both and died. The rapid development of coma after a violent tetanus we will discuss later.

Group VI. Frog A, injected normal bile 2 c.c. at 10.30 A.M. At 2 P.M., reflexes increased. 6 P.M., normal.

Frog A′, injected normal bile 2 c.c., then 0.01 mgr. strychnine at 10.30 A.M. At 2 P.M., marked increase of reflexes, rigidity on handling; next day normal.

Frog B, injected normal bile 3 c.c. at 10.30 A.M., at 2 P.M., stupor and paresis; at 6 P.M., coma and paralysis; next day dead.

Frog B′, injected normal bile 3 c.c., then 0.01 mgr. strychnine at 10.30 A.M. 2 P.M., normal; 6 P.M., normal; remains normal next day.

Frog C, injected normal bile 5 c.c at 11 A.M.; 2 P.M., coma and paralysis; 6 P.M., dead.

Frog C′ injected normal bile 5 c.c., then injected 0.01 mgr. strychnine at 11 A.M.; 2 P.M., coma and paralysis; 6 P.M., dead.

In this experiment the addition of strychnine to two cubic centimeters of bile brought rigidity, while the control animal remained normal; and the addition of the same amount of strychnine to three cubic centimeters of bile brought about normal conditions while the control died from coma and paralysis. Finally, the addition of the same amount of strychnine to five cubic centimeters of bile did not reduce the paralyzing effect of a large dose of bile, and there was practically no difference between the frog given strychnine and the control. The addition of strychnine brought out then first tetanic symptoms; when the quantity of bile and with it the strength of the depressing element were increased, the antagonistic elements were just sufficient to neutralize one another. When, however, the depressing element was increased considerably, it not only neutralized all tetanic effects but had a sufficient balance left to produce paralysis and coma.

These striking results were, however, rarely met with. They would seem to depend upon so many circumstances more or less uncontrollable as to make their intentional or even accidental production infrequent. But instances of mutual neutralization of less striking character were much more frequently produced.

The addition of ineffective doses of strychnine to the bile of nephrectomized rabbits brought out instructive observations. From the results in Series IV, we had reasons to surmise that a certain amount of a tetanic element of metabolic origin, which is normally eliminated through the urine, passed in nephrectomized rabbits through the bile. We compared in a number of experiments bile from nephrectomized rabbits with that of normal animals as to the effects of the addition of strychnine. In the majority of experiments the difference was very strikingly in favor of the bile from nephrectomized rabbits being rendered more tetanic than normal bile by this addition. Some of the experiments follow:

Group VII. Frog A, injected at 3 P.M. 3 c.c. of normal bile, and then at 3.25 P.M. 0.01 mgr. strychnine. No signs of tetanic effect; paralysis; dead next day.

Frog A′, injected at 2.30 P.M. 3 c.c. bile from nephrectomized rabbit, and at

3.20 0.01 mgr. strychnine. At 3.40 legs rigid, later tetanus on handling; normal next day.

Frog B, injected 5 c.c. normal bile at 2.40 P.M, and at 3.25 added 0.01 mgr. strychnine. No signs of a tetanic influence. Coma, paralysis, and death.

Frog B′, injected 5 c.c. of bile from nephrectomized rabbit at 2.40 and at 3.25 added 0.01 mgr. strychnine. At 4.25, distinct rigidity on handling, and at 5, distinct tetanic attacks. Normal next day.

Group VIII. Frog A, injected at 12.30 P.M. 5 c.c. normal bile, and immediately after 0.01 mgr. strychnine; 2.30, coma and paralysis; dead next day.

Frog A′, injected at 12.30 P.M. 5 c.c. of bile from nephrectomized rabbit, and immediately after 0.01 mgr. strychnine. 2.30, increased reflexes and rigidity of extremities; no coma; next day reflexes still increased, and on hitting table, mild tetanus. Survived.

In these and in some additional experiments it was shown that the addition of ineffective doses of strychnine to comparatively large doses of bile of nephrectomized rabbits produced tetanic symptoms, while the same doses of strychnine and normal bile produced coma and paralysis. In other words, nephrectomy apparently increased the tetanic action of the bile.

We have up to now dwelt chiefly on our observations upon the increase of the tetanizing effects of bile by the addition of ineffective doses of strychnine. We have now to state that under certain conditions the addition of strychnine favors the appearance of the depressing effect of bile. Indeed among the experiments described above were some in which this action of strychnine in favoring the appearance of coma and hastening the death of the frogs was shown. This effect has been noted by us in the case of the bile from a large number of rabbits as well as other animals. These observations may be summed up as follows: When in employing doses of bile which, when injected alone, never produced any degree of coma and paralysis, there was added strychnine, no matter whether in small or large doses, it never favored the appearance of the depressing phenomena; if, however, the bile caused alone the appearance of coma and paralysis quite readily, and the dose employed was one producing sooner or later some degree of coma and paralysis, then the additional injection of strychnine frequently hastened the onset and increased the rapidity of development of the depressing effects In some cases the sudden onset of coma was preceded by very

brief and rarely by very violent tetanus. The tetanus was usually so brief that it may in other instances have frequently been overlooked. The larger the added amount of strychnine, the more quickly the tetanus and the sconer complete coma and paralysis would set in.

In cases where coma and paralysis are brought on by bile alone, the development of the depressing symptoms takes place gradually and slowly, a fact especially true of doses not too large. The usual course is as follows: After the animal recovers its activity immediately after receiving bile it gradually becomes abnormally quiet and remains sitting in one place with the head gradually sinking down to the table. At this stage handling arouses it for a while and it jumps away in a normal manner, but as soon as left alone it becomes again droopy. Soon it offers little resistance, very often it does not jump away, and when it does jump it does so clumsily for short distances. When turned on its back it makes only feeble attempts to turn on its stomach. However, once in a while it succeeds, and when again on its stomach draws its legs near the body, the head and stomach now resting on the table. Gradually the limbs become limp and in the attempts to move they get away from the body; their tonicity is lost and they look œdematous, after which the animal soon appears to be perfectly paralyzed. Occasional sudden, feeble twitchings of some parts of the body occur, the respiration becomes feebler and slower, the heart-beats become diminished. At this stage the pupils are generally constricted. The frog can remain in this condition for many hours before all signs of life disappear. Between the injection and the state of complete coma and paralysis many hours often elapse.

The course is entirely different when in addition to an effective dose of bile a dose of strychnine, especially one approaching the effective minimum, is given. On being put under the glass bell the animal becomes very lively, keeps its head high, and is continually moving around; it is always near the glass wall looking for opportunities to escape. On hitting the table suddenly or handling the animal, and sometimes even without any visible cause, the frog is seized with a tetanic convulsion and a few

seconds later is lying stretched out as nearly paralyzed as a frog in advanced stage of depression from bile alone. A blow on the table may still bring on brief responses of feeble stretching of the extremities, but in a short time there is no visible difference between the paralysis of the strychnine frog and the paralysis brought on simply by the bile.

As to the cause of this paradoxical effect of strychnine, there are several possible explanations. We may think in the first place of the possibility that strychnine, containing as is well-known a certain depressing element, manifests this action after the tetanus is over, and hence we might assume that it is this depressing effect added to that of the bile which hastens the onset of complete depression. It is probable also that the sudden onset of coma is always introduced, as we have already mentioned above, by a brief tetanus which on account of its slightness is frequently overlooked. We may then assume further that after the convulsive explosion, the resistance of the tetanic element becoming momentarily exhausted, the depressing phenomena appear suddenly. Finally, the normal interval between the injection of the bile plus the strychnine and the outbreak of acute coma is upheld by a very unstable equilibrium between the two antagonistic elements. The sudden outbreak of the tetanic condition disturbs this equilibrium, with the result that henceforth the stronger factor, in this instance the depressing element, dominates the field. It is perhaps possible that the acute outbreak is brought about by a combination of all the factors mentioned in the three possibilities.

We have stated that after the outbreak of the coma and paralysis the frogs remained in a state of depression until death. This is the usual outcome. In a few instances, however, the course up to the acute onset of the coma was the same as in all the other experiments. The further part of the course, however, was strikingly different. The animal would lie apparently in complete coma and paralysis; but a stimulus of any kind—such as touching the frog or striking the table—would bring on a pronounced tetanic attack of short duration which would pass rapidly into complete coma and paralysis. Sometimes these

tetanic attacks would break out apparently spontaneously. This alternation between tetanus on the one hand, and coma and paralysis on the other, would continue for many hours, the condition reminding one in a striking way of the syndrome in human pathology designated "eclampsia."

The quantity of strychnine employed in these cases had to be at least near the minimum effective dose; the quantity of bile, on the other hand, had to be not too much above the minimum dose which when employed alone brought on coma and paralysis. When large doses of depressing bile were injected no such effect could be brought on by the addition of even a large dose of strychnine.

The tetanus occurring in these "eclamptic" frogs was markedly in contrast with the tetanus brought on by bile alone. Each single tetanic attack in the latter cases lasted a long time, and after passing off left behind a marked tonicity of the muscles, giving to the animal a somewhat "graceful" appearance. Each tetanus in the "eclamptic" state was very brief and passed abruptly over into perfect relaxation, which gave the animals an "œdematous" appearance.

Finally, we have to add that, when after the injection of bile coma and paralysis were already completely developed, the injection of strychnine even in doses of 0.04 or 0.05 milligramme hardly ever exerted any influence.

The paralysis which bile produces is in the first place of central origin. This fact has been demonstrated by Rywosch and others by direct experiment. We repeated one of the experiments. In a completely paralyzed animal which received the bile injection, electrical stimulation of the cord produced no effect upon the muscles of the trunk or the extremities; while stimulation of the sciatic nerve produced quite active contractions of the extremities.

That bile also affects nerve and muscle directly was shown long ago by many observers. In our experiments we frequently had occasion to observe this effect in the following manner: In order that the large quantities of bile which we introduced should not escape again, we injected the lymph-sacs by passing

the needle through the muscles of the thigh, the muscles thus working as a valve. Now these legs, and especially the thigh, were often paralyzed long before there was any sign of paralysis in the rest of the frog. In some instances the frog would respond with a tetanus in which even the lower part of the paralyzed extremity would take part. The paralysis of the thigh in those cases was caused undoubtedly by the bile coming in direct contact with the muscles of the thigh. This would mean that even that bile which failed to produce paralysis through the central nervous system might still reduce the irritability of the muscles by coming in direct contact with them.

The reduction of the irritability and conductivity of nerves and muscles by bile had probably some share in the advanced stages, and possibly also in the earlier stages, of paralysis. Of the latter we had to think especially when the injection of bile was given into the lymph-sacs, while the animal remained in its normal position, as was the rule. It might thus happen that some bile reached the sciatic plexus or nerves and caused a premature local paralysis of the posterior extremities. Indeed we met with these examples in cases in which ineffective doses of strychnine in addition to bile were employed, and in which the front legs became rigid while the posterior extremities were paretic or paralytic. In a few cases, therefore, immediately after injecting the bile into the lymph-sacs, we have suspended the frogs by the legs, causing the bile to accumulate preferably in the anterior parts of the body. In these experiments it seemed, indeed, that the addition of strychnine brought on rigidity of the posterior extremities very readily and distinctly, while the animal seemed otherwise to be in a state of stupor.

We should mention further that of the rabbits' bile which we have employed some was received sterile, some was sterilized, and some was exposed to boiling for half an hour before it was used. A few of the experiments with the last bile made the impression that boiling reduces the activity of the depressing element and favors the appearance of tetanus. However, the analysis of all the experiments does not positively justify such a claim. It may be stated with safety that boiling does not affect

in a pronounced manner either of the biliary elements described. But in a number of comparative experiments it was shown that boiling reduces the toxicity of the bile as a whole. The animals which received boiled bile survived pronounced attacks of tetanus as well as some conditions of coma and paralysis, whereas all the control animals with the same amount of unboiled bile, which otherwise produced the same symptoms, died a day or two after the injections. Our experiments were not extensive enough in this regard to permit a more definite statement.

The numerous facts communicated in the foregoing pages were derived, as has frequently been stated, from the study of rabbits' bile. We also stated that the toxicity of the bile of other animals and of bile salts was studied. These latter studies were not as full as the other and were restricted to ascertaining a few essential facts. The results of these studies will now be reported briefly.

Guinea-Pigs' Bile.—The bile of only three guinea-pigs obtained from a cannula fastened either in the common bile duct or in the gall bladder was at hand. The quantity yielded was small and our observations therefore are limited to twelve frogs.

Two or even three c.c. of fistula bile caused no toxic effects of any kind. One c.c. of stagnated bile from the gall bladder also had no effect. The addition of 0.01 mgr. strychnine did not bring on hyperæsthesia. The addition of 0.02 mgr. strychnine brought on, after many hours, distinct rigidity, while the control animal remained normal.

These few results are recorded without attaching much importance to them. The observations were too few and too little varied to permit positive conclusions. It may be said, however, that, few as they were, they were more numerous than some from which certain writers drew their conclusions regarding the nature of the toxicity of bile.

From the fact that even three cubic centimeters were without toxic effect we were at first inclined to the general conclusion that guinea-pigs' bile is less toxic than rabbits', but this notion was dissipated by the experiments with rabbits' bile in which the toxic dose was definitely greater than this amount. It will take a more extensive study to decide whether guinea-pig's bile is indeed less toxic than rabbit's.

Dogs' Bile.—Our experience with dogs' bile is also limited. The few experiments made, gave, however, some definite results in accord with those obtained with rabbits' bile.

Group IX. Frog 1, control, injected 0.01 mgr. strychnine at 3.50 P.M. No effect.

Frog 2, injected 0.5 c.c. boiled dogs' bile (from fistula) at 3.50 P.M. No effect.

Frog 3, injected 0.5 c.c. boiled dogs' bile followed by an injection of 0.01 mgr. strychnine at 3.50 P.M. At 4.30, exaggerated reflexes followed soon by typical tetanus.

In this experiment 0.5 of the bile had no effect, but the addition of an ineffective dose of strychnine brought out a typical tetanus. That means that 0.5 of the dogs' bile contained a tetanic element large enough to convert a subminimum dose of strychnine into a toxic one.

Group X. Frog 1, weight, 36 grammes; injected 1 c.c. of dog's bile at 10.15 A.M. At 12 M., complete coma and paralysis.

Frog 2, weight 42 grammes; injected 1 c.c. of dogs' bile and immediately afterwards 0.01 mgr. strychnine at 10.15 A.M. At 10.50, complete coma and paresis. 12 M., dead.

One c.c. of dogs' bile caused complete coma and paralysis in about two hours; the addition of strychnine hastened the onset of the depressive phenomena and death, an experience similar to that frequently seen in case of the rabbits as described.

Hyperæsthesia and convulsions did not occur after injections of dogs' bile without the addition of strychnine. Still our experience with this bile was too small to permit the drawing of any conclusion.

Ox Bile.—Ox bile as it was obtained from the slaughter-house and inspissated bile dissolved in various concentrations were employed. The bile frequently was filtered and sterilized before using. Our experience with this bile was quite extensive, and hence the few results reported were conclusively established. It is desirable to keep in mind when comparing the effects of ox and rabbit bile that all of the former was obtained from the gall bladder.

Ox bile is very toxic; 0.3 c.c. will, as a rule, cause sooner or later toxic symptoms and death. The former are nearly

exclusively depressive, namely: coma and paralysis. In all our experiments we came across tetanus once only. This frog received 0.3 c.c. of bile; first developed coma; but the next day the coma was interrupted by tetanic attacks—in other words, an eclamptic state supervened. Smaller doses never gave even hyperæsthesia. Since this effect is similar to that obtained with rabbits' bile taken from the gall bladder or from the dilated common duct, stagnated bile is apparently very toxic, the depressing element being the controlling factor in it. We observed, however, numerous proofs of the existence of a tetanic element even in this depressing bile.

Group XI. Frog A, received 1 c.c. of a 10 per cent. solution of ox bile. Condition good; no effect.

Frog A', received 1 c.c. of a 10 per cent. ox bile followed by 0.005 mgr. strychnine at 4 P.M. At 6 P.M., increased reflexes.

Frog B, received 1.25 c.c. 10 per cent. ox bile; condition remained good.

Frog B', received 1.25 c.c. 10 per cent. ox bile followed by 0.005 mgr. strychnine at 4 P.M. At 5 P.M., violent tetanus.

In this series neither the doses of bile employed alone nor strychnine alone produced any effect. When, however, both were given, the effects produced were hyperæsthesia and sometimes tetanus.

Moreover, in some instances when the toxic dose of bile was on the border line and tended sooner or later to bring on depressing symptoms followed by death, the addition of strychnine still brought out tetanus. Of this class of effects the next experiments form examples.

Group XII. Frog 1, injected 0 25 c.c. ox bile at 11.30 A.M. At 3 P.M., marked stupor. Next day, dead.

Frog 2, 0.25 c.c. ox bile, followed by 0.01 mgr. strychnine injected at 11.30 A.M. At 3 P.M., slight stupor. Next day, distinct tetanus.*

On the other hand, we observed many instances in which a dose of 0.01 milligramme strychnine or more, when superadded to a distinctly toxic dose of ox bile, hastened the onset of the depres-

* Such effects remind one of the action of certain doses of morphine upon frogs. On the day of injection there is either no effect or a moderate stupor, while a day or two later a distinctly tetanic condition develops.

sive symptoms and death. In some of these the coma, which was brought on acutely by the addition of strychnine, was not of a continuous, but rather of an "eclamptic" character.

There were some slight variations in the toxicity of the bile obtained at different times. Whether this variation was due to individual differences in the animals we cannot state, since we are not sure that each portion of bile was obtained from a single animal.

Ox bile is therefore highly toxic in small doses and exceeds in toxicity gall bladder bile of rabbits; but like the latter its effect is essentially depressive. The addition of ineffective doses of strychnine shows, however, that ox bile contains an effective tetanic element, the manifestation of which is suppressed by the superior antagonistic effect of the depressing agents. In other words, by the addition of strychnine to ox bile all the phenomena observed in the study of bile from the common duct of rabbits plus strychnine were produced.

Bile Salts.—Glycocholate and taurocholate of sodium were studied separately. According to Rywosch,[19] the fatal dose of sodium taurocholate for frogs weighing from 40 to 50 grammes is about 60 to 70 milligrammes, and of sodium glycocholate about 100 milligrammes. Judging by his protocols Rywosch had in mind doses which produced coma and complete paralysis in less than half an hour and death in less than two hours. In most instances the frogs which we used weighed between 30 and 35 grammes. We considered the bile salts toxic when a given dose produced pronounced toxic symptoms within a few hours. These experiments may be summed up briefly as follows:

A few frogs responded to injections of 20 milligrammes of bile salts, and more responded with distinctly toxic symptoms and a fatal result on the day of injection, or within the next few days to 30 milligrammes. In some instances even 40 milligrammes were without positive effect, and exceptionally some effect was obtained with 15 milligrammes. Fifty milligrammes or more were invariably highly toxic.

The toxic symptoms were stupor and complete coma, paresis,

[19] Rywosch, l. c.

and complete paralysis. Beginning with small quantities and gradually increasing the doses of the bile salts until definite and fatal symptoms set in, we came across only one instance for each salt of a tetanic effect of doubtful character. The exceptions were as follows: With a dose of sodium glycocholate of 40 milligrammes, there were no perceptible symptoms on the same day, but on the next day some tonic rigidity seemed to be present. In another instance about half an hour after injection of 25 milligrammes of sodium taurocholate coma developed, and while handling the frog a slight rigidity set in which soon passed over into complete paralysis.

These two doubtful instances of temporary rigidity were the only signs of the presence of a tetanizing influence among the experiments with the bile salts. There was in this regard no perceptible difference between the effects of sodium taurocholate and glycocholate. It should also be added that in our experiments no difference in the toxicity of the two salts was noticed, a point of difference with Rywosch.

The experiments with the addition of strychnine did not bring out any striking results which favor the notion of the presence of a tetanic element in the salts. Less than 0.01 milligramme had not the slightest effect. Of many experiments with additions of 0.01 milligramme of strychnine to the dose of sodium glycocholate, in one only was slight hyperæsthesia noted. This amount of strychnine when added to sodium taurocholate (10 mgr. salt + 0.01 mgr. strychnine, mixed) produced in one case, after $2\frac{1}{2}$ hours, a passing tetanus, and in another case (30 mgr. + 0.01 mgr.) a slight tetanus. In the many other experiments with the taurocholate (strychnine 0.01 and 0.015) there was no effect except perhaps a slight hyperæsthesia in one or two instances. The addition of 0.02 milligramme of strychnine to sodium glycocholate brought out tetanus in two experiments, while the control with strychnine alone gave only hyperæsthesia; in other instances there were only increased reflexes. We had the same experience with sodium taurocholate.

In a few instances the addition of a larger dose of strychnine hastened the onset of coma. Two of the above-mentioned at-

tacks of tetanus were the precursors of coma and paralysis which continued until death, except in two examples where the course was of a typically "eclamptic" character.

The general conclusions which our experiments with bile salts permit us to draw are that the toxic effects of the salts upon the general nervous system are essentially depressing in character. The salts probably also contain the tetanic element, but in very small amount, hence the addition of strychnine is distinctly less effective than even with bile which comes from the gall bladder.

In reviewing our results we shall trace our steps backwards. The investigation, which we began in the attempt to solve our original problem, led us to the question of the actual nature of the toxic action of bile. As an answer to this question we obtained a number of facts the signification of which we wish to present.

It is now generally accepted that the toxic effects of bile are only of a depressing character. In our studies we found this to be true for the bile obtained from the gall bladder of the rabbit and ox, at least as far as the actual effect is concerned. In a few instances, in which we made experiments, this fact held good for the gall-bladder bile of dogs and guinea-pigs. Sodium taurocholate and glycocholate also proved to have a depressive action.

It was, however, different with bile obtained from a fistula of the common duct of rabbits. The bile of certain rabbits produced hyperæsthesia and true tetanus, but never coma and paralysis. The bile of other rabbits produced marked hyperæsthesia with small doses, and coma and paralysis with large ones. Only in a few experiments did this bile cause coma and paralysis and no increase of excitability. Hence we have positive and direct proof that normal bile from many rabbits possesses an exciting element capable of producing clonic and tonic convulsions in frogs.

We found that small doses of bile of any kind could cause hyperæsthesia and tetanus when supported by small doses of strychnine. The doses of the bile and strychnine which come

into play in these experiments were below that which separately produced symptoms. This could only mean that bile contains an element which like strychnine can produce increased excitability, and which can supplement it. We designate this hypothetical agent in bile for sake of brevity as the tetanic element. By the same method we have demonstrated the existence of a tetanic element in the bile of the common duct of rabbits which alone gave only depressing symptoms, and in the gall-bladder bile of dog, ox, and rabbit. The evidence obtained for the presence of the tetanic element in bile salts was not conclusive. If present at all, it apparently occurs in smaller quantity than in the whole bile.

We may, therefore, claim from our experiments that we have established the fact that bile from the common duct of some animals, at least, contains a tetanic element which may manifest its presence without the aid of strychnine; and, in some instances, would appear to be the sole toxic agent present.

Gall-bladder and stagnant bile of all animals produce depressing symptoms, although they surely contain a tetanic element.

From Series II and III it would appear that bile which is obtained soon after the preparation of a fistula is apt to cause depressive symptoms chiefly.

In some animals indeed all the bile from the common duct causes in comparatively small doses only coma and paralysis. It should be added that the flow of bile in these animals was slow, and the quantity collected was small as compared with that obtained from animals the bile of which produced hyperæsthesia and convulsions. All these facts considered, it may be stated that stagnant bile produces depressing symptoms.

Our experiments have demonstrated not only that all kinds of bile contain the tetanic element, but also that this and the depressing element are present simultaneously. In stagnant bile, though it surely contains both elements, it is, apparently, the depressing element essentially which manifests its effects. What are the mutual relations of these two toxic agents? From our experiments with the addition of strychnine we may answer this very difficult question as follows:

1. That the depressing effect of large doses of bile cannot be reduced or visibly modified by the addition of any dose of strychnine.

2. That the depressing effect of doses of bile, in quantities near the toxic minimum, can be promptly modified by a sub-minimum dose of strychnine: (a) The effect can be neutralized or annihilated, whence a normal condition results; (b) the effect can be overcome to such an extent as to bring out the opposite or tetanic effect.

3. That by an increase of the depressing element the positively tetanic effect of strychnine plus the tetanic element in the bile can (a) be neutralized, i. e., reduced to normal, or (b) made to produce coma and paralysis.

These observations lead us to assume in the first place:

That the depressing and tetanic elements are exact antagonists and that their joint effect is that of an algebraic sum.

That, furthermore, when each of the antagonists is simultaneously present in the bile in a distinctly effective minimum, the effect of the depressing element is by far the stronger, so as to conceal any modifying effects of its antagonist.

That the tetanic element attains its minimum effectiveness in a much smaller dose than is required for the effective minimum of the depressing element.

On the basis of these interrelations we can explain the following combinations:

When the depressing element is present in a distinctly effective minimum, the result of an intoxication will always be coma and paralysis.

When the tetanic element is present in a strength which is its minimum or above it, while the depressing element is far beneath its own minimum, the result will be hyperæsthesia and possibly tetanus.

When both elements are present in minute quantities, very small doses of the bile will be harmless. With an increase of dose a stage will first be reached in which the tetanic element will attain its minimum, but reduced somewhat in effect by a low strength of the depressing element, a condition leading to

hyperæsthesia. The next increment of increase of dose will be influenced more by the depressing than by the tetanic element, through which the result may be the production of a normal condition, due to exact mutual neutralization. Any further increase of dose will, of course, lead to supremacy of the depressing element. The middle stage, which depends largely upon exact neutralization, will frequently be missed or overlooked.

The assumption that the minimum for the tetanic element lies much lower than for the depressing element,[20] and the fact that the definite minimum of the depressing element always overpowers the tetanic element, readily explain why in stagnant bile the depressing effect is supreme.

Let it be assumed for the sake of illustration that D (depressive) = 2 T (tetanic), and let it be further assumed that 4 c.c. contains D + 2 T, then 4 c.c. will give coma and 2 c.c. ($\frac{1}{2}$ D + T) will give tetanus. When the stagnant bile loses half of its water without losing its toxic elements, then 2 c.c. of this bile will contain D + 2 T, with the result that now 2 c.c. of this bile will cause coma and paralysis instead of tetanus.

In all of these considerations we have however to remember that in the algebraic sum of both antagonists, no matter how small one of the factors may be, none of the effect is actually lost, i. e., if the result is coma and paralysis in some degree, we have to think were it not for the simultaneous presence of the tetanic element the degree of depression would have been higher; and vice versa.

The conditions and relations which we have just described hold good for stable relations only, i. e., when both antagonistic elements exist in such firm relations to one another as to produce a state of equilibrium. When these mutual relations are disturbed, as would for instance happen when one or the other element was suddenly superadded in a certain strength, the outcome would be a struggle for supremacy, which might continue or be early decided for the one side or the other, whence the equilibrium would be broken. We have seen this

[20] An analogy is found in the minimum toxic dose of strychnine (tetanic), which is 10 times lower than the minimum of morphine (depressive).

happen in our experiments where the addition of an effective dose of strychnine to an effective dose of bile brought out a very short tetanic effect, which immediately gave way to a premature onset of deep coma and paralysis. The depressive phenomena were either continuous till premature death, or an alternating change between coma and convulsion, or a state of "eclampsia" occurred.

Finally, we have to point out that in bile salts the tetanic element is apparently present in less amount than in the whole bile. This fact does not agree well with the now generally accepted theory that the entire toxicity of bile resides in the bile salts. An assumption that the tetanic element is intimately associated with some other portion of the bile, not yet isolated in purity, would seem more in harmony with the facts; in which case it might be thought that only a fraction of this element clings to the bile salts. It should also be recalled that in our experiments the tetanic element was not destroyed by prolonged boiling of the bile. Still we do not wish to draw final conclusions from our series of experiments with bile salts.

The results of the series of experiments which we have just analyzed demonstrated that bile from the common duct of normal animals does contain a tetanic element and produces occasionally convulsions, and they also demonstrated that the addition to this bile of a tetanic factor of any source will increase the tetanizing efficiency of that bile. We shall now examine in the light of the last experience the results which we obtained in the first few series of experiments.

In Series IV we found that the bile from the common duct of nephrectomized rabbits showed a tetanic character more frequently than the bile of normal animals in Series V, and much more so than the bile of the ante-strychnine period in Series II (nephrectomized rabbits). We can now safely interpret this observation on the basis of the assumption which we previously suggested, namely, that normally a tetanic agent of catabolic origin is being eliminated in some degree through the urine.

After nephrectomy this tetanic agent finds its way, in part or

wholly, into the bile and thus increases the efficiency of the tetanic agent normally present in this fluid.

This interpretation is also in harmony with the observation that the addition of strychnine to bile from nephrectomized rabbits increases the tetanizing effect of this bile more markedly than bile of normal rabbits.

But as the bile of the ante-strychnine period in Series II is, as we stated above, more or less stagnant, the prevalence of depressive symptoms of intoxication in spite of the added tetanic element is easily understood.

The bile of the post-strychnine period of Series II appeared to be more tetanic than that of any other series. We assume that in this series some at least of the strychnine was eliminated through the bile. Hence the tetanic agents in this bile were of three kinds: the tetanic element of the bile itself, the strychnine, and the tetanic element ordinarily eliminated through the urine. Through the united effort of all the three agents, the effects of the antagonistic depressing element are considerably overcome; whence the considerable increase of the exciting effects of the bile of this series.

The same reasoning applies to the bile of at least two normal animals of Series III which received strychnine. An instructive incident is the observation in these cases that the urine of these animals had nearly no hyperæsthetic effect, while in normal animals which receive strychnine the urine, as a rule, contains so much strychnine as to produce tetanus. Our explanation of the underlying conditions is as follows: Normally strychnine is eliminated through the bile into the intestines, but only to be reabsorbed and to be gradually and finally eliminated through the urine. However, as the bile is continually being discharged outside of the body through a fistula from the common duct, there is less chance for the strychnine to get into the urine.

In the first series of nephrectomized rabbits with ligated common duct the strychnine had diminished avenues of escape from the body, whence the proportionately more frequent occurrence of tetanus in these animals than in animals which were nephrectomized and then received subminimum doses of strychnine.

Furthermore, it is possible that in some cases strychnine accumulated in the gall bladder, the biological test failing to demonstrate its presence on account of the overpowering effect of the depressive element in this stagnant bile.

RESUMÉ.

The preceding investigation can be summarized as follows:

That, contrary to the prevailing opinion, bile contains a tetanic element, or an agent which causes increase of excitability of the nervous system.

That bile from the common duct of many rabbits causes marked hyperæsthesia and tetanic attacks when injected into frogs.

That stagnant bile (as in the gall bladder, etc.) invariably produces coma and paralysis.

That the depressive and exciting elements of the bile are mutual antagonists, the total effect of both, when simultaneously present, being that of an algebraic sum.

That the depressive element when present in a highly effective dose is by far the stronger of the two; while, on the other hand, the tetanic element becomes effective apparently in a dose far below that which constitutes the minimum for the depressive element.

That owing to these peculiar characteristics of the two antagonistic toxic elements, the mixture of both within the bile can be so adjusted as to produce any of the many possible degrees of irritability lying within the range of tetanus at one end and paralysis at the other, and including a neutral state presenting apparently a normal or tranquil condition.

That bile salts seem to contain the tetanic element in a distinctly less amount than the whole bile.

That nephrectomy increases the toxicity of bile in the direction of excitation.

That the effect of nephrectomy can be imitated by subcutaneous injections of strychnine.

That the bile of nephrectomized rabbits which received

strychnine produces hyperæsthesia and convulsions more readily than any other bile.

That, finally, by injecting a toxic dose of strychnine into a frog soon after it has received a distinctly depressing dose of bile, an "eclamptic" state can in some cases be produced.