THE ACTION OF HEPATIC, RENAL AND OTHER CELLS ON PHENOL AND INDOL, UNDER NORMAL AND PATHOLOGICAL CONDITIONS.*

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It is the object of this communication to give the results of experimental observations on the behavior of various animal cells toward certain substances belonging to the aromatic type, notably phenol and indol. The inquiry was prompted by the interest which attaches to a study of the natural defenses of the organism against various kinds of damage through chemical agencies. Prominent among the problems that arise in connection with such an inquiry are, first, the determination of the seat of the defensive action of the organism, whether chiefly in the blood or in the cells; second, the relative activity of the different kinds of cells in the neutralization of the toxic properties of the particular chemical agents employed; and third, the character of any chemical transformation that may take place, when these substances are acted upon by living cells.

Indol and phenol were selected for use in the inquiry for two reasons. First, these substances are normal products of proteid cleavage in the intestine, under the influence of bacteria and of the proteolytic ferment of the pancreatic juice, and they are often formed in excessive amounts in the course of digestive derangements. Greater interest thus attaches to the fate of these substances in the organism than to that of wholly foreign poisons. Second, both indol and phenol are recognizable by means of delicate color reactions, and it becomes possible by means of the procedures to be described to make use of these color tests for the purpose of comparing the relative proportion of phenol and indol present in different solutions.

The Contact Method.—Two methods were pursued in our observations. In the first, the organs of healthy rabbits were quickly removed, after

* Read before the Association of American Physicians, May 4, 1899.

bleeding nearly to the point of death. After chopping the organs into fine bits, seven grammes of the pulp of the liver, kidney, brain muscle, and seven grammes of the blood were brought into contact with ten cubic centimetres of a weak solution of phenol and indol of known strength. The period during which these solutions were left in touch with the organ pulps varied in different experiments, two or three hours being the usual period. At the end of this time the mixture was subjected to distillation. The distillate was then tested for the presence of indol by the nitroso-indol test, and for phenol by means of Millon's reagent. By placing in columns equal heights of distillates in test tubes it becomes possible to arrange these in the order of the intensity of their color. Two or more members of the series sometimes give colors of equal intensity, or the difference may be so small that it is difficult to be certain of any difference. Usually, however, a good gradation of color can be obtained throughout the series. It is hardly necessary to state that the utmost care in technique is necessary in making these observations, by what we have come to call the "contact method." Under carefully controlled conditions it is possible to obtain reliable and consistent results.

It will be seen by reference to Tables I and II that the different organ pulps possess in different degrees the power of causing the disappearance of the phenol or indol with which they are brought in relation.

That all of them possess some activity, is seen by the fact that their distillates yield less color than a blank control of the solution, which has also been subjected to distillation. We may refer first to the results obtained with phenol and then to those with indol.

Order of Activity of Different Cells.—Of the twenty-seven observations on phenol, it is seen that the liver distillate gave the smallest amount of color in all twenty-seven cases, *i. e.*, in all instances the activity of the liver in disposing of phenol was greater than that of any of the remaining organs studied. In Table I in which the organs are arranged in series, according to the color reactions of their distillates, this fact is clearly brought out by the circumstance that all the livers are to be found in the right-hand column. The table shows further that the kidney occupies the next place to the liver as regards activity. Of the twenty-six cases in which the kidney was studied it comes into the column next that of the liver in eighteen. In the next

column the muscle occurs sixteen times. In the next column to the left the blood is found in twelve cases. In the next column to the left the brain is found eleven times. Finally, in the first column are seen all the control observations made upon blank solutions of phenol like those that we used in the contacts. In most instances it was possible to obtain a gradation of color. Where different tissues gave the same tint, this is indicated by the line drawn under the letters designating these tissues.

TABLE I.—EXPERIMENTS WITH PHENOL. CONTACT METHOD.

No. hours contact.						
3	Blank	Bn	Bd	M	K	L
3	"	Bd	Bn	M	K	L
3	"	Bn	Bd	M	K	L
3½	"	Bn	Bd	M	K	L
4	"	Bd	M	K	Bn	L
3½	"	Bd	M	K	Bn	L
3½	"	Bn	Bd	M	K	L
3½	"	M	Bd	Bn	K	L
3	"	Bn	Bd	K	M	L
3	"	Bd	Bn	M	K	L
3	"	Bn	Bd	M	K	L
3	"	Bd	Bn	M	K	L
3	"	Bn	Bd	M	K	L
3	"	Bd	K	Bn	M	L
2	"	Bn	Bd	M	K	L
21	"	—	Bd	M	K	L
Few minutes	"	Bn	Bd	M	K	L
6	"	—	M	Bd	—	L
3	"	Bd	Bn	M	K	L
2	"	Bn	Bd	M	K	L
2	"	Bn	Bd	M	K	L
About 2 hours.	"	Bd	Bn	M	K	L
3	"	Bn	Bd	M	K	L
3	"	Bd	Bn	K	M	L
1	"	Bd	Bn	M	K	L
3	"	Bn	Bd	M	K	L
3	"	Bd	Bn	K	M	L

Bd=Blood, Bn=Brain, M=Muscle, K=Kidney, L=Liver.

Thus it is evident that in all instances the blood, brain, muscle, kidney and liver exerted some influence in transforming a certain amount of phenol so that it no longer responded to Millon's reagent. The results show very clearly that in point of activity the liver leads, with the kidney a close second and the muscle third. The blood and brain occupy the fourth and fifth places, although they are both less active than the muscle. The brain and blood are much alike in their behavior and appear to exert little action on the phenol. On the other hand, the action of the liver is often sufficient to yield only a very feeble color reaction with a solution of the strength employed.

Looking now at the results in the case of indol (Table II) it is seen by the table that here there is less uniformity than in the case of phenol. There are thirty observations in all, but in many of these a gradation of color through the series is impossible, so that many ties are noted in the table. Taking the liver first, it is seen that there are eleven cases in which it leads in activity. Twice it is tied with the kidney, once with the brain, twice with muscle and brain, twice with kidney and brain, and once with kidney, muscle and blood. In spite of these ties, however, it is clear that the liver leads the other organs in activity. But the kidney is a very close second and is found five times in the column indicating the greatest activity.

Without going into further detail, it may be stated that notwithstanding numerous irregularities, the table indicates that the order of activity of the cells acting on indol, is as follows: liver, kidney, muscle, brain and blood. Between the activity of brain and muscle there seems little difference.

In one respect these results differ from those obtained in the case of phenol,—the blood shows the smallest action of any member of the series, a degree of activity apparently less than in the case of phenol.

Isolated trials of the activity of the epithelium lining the small intestine of the rabbit indicate that these cells possess a high grade of effectiveness, comparable to that of liver cells. The cells of the spleen pulp are similarly but somewhat less active. Observations were also made with potato in order to ascertain whether the properties noted in animal cells pertain also to vegetable tissues. The results indicate a

moderate degree of activity on the part of potato in transforming phenol. Similar trials with egg albumen showed absolutely no action on the part of the proteid material. Experiments with gelatine gave entirely negative results.

TABLE II.—EXPERIMENTS WITH INDOL. CONTACT METHOD.

No. Hours Contact.						
2	Blank	Bn	Bd	M	K	L
3	"	Bd	M	K	Bn	L
3	"	Bd	Bn	M	K	L
2	"	Bn	Bd	M	K	L
4	"	Bd	Bn	M	K	L
20	"	Bd	L	Bn	M	K
20	"	Bd	M	Bn	K	L
4	"	Bd	M	Bn	L	K
4	"	Bd	Bn	M	L	K
4	"	Bd	Bn	M	L	K
4	"	M	Bd	Bn	K	L
4	"	Bd	M	K	Bn	L
3	"	Bn	Bd	M	K	L
3	"	Bn	Bd	M	K	L
4	"	Bn	Bd	M	K	L
20	"	Bd	L	Bn	M	K
20	"	Bd	M	Bn	K	L
4	"	Bd	M	Bn	L	K
4	"	Bd	Bn	M	L	K
4	"	Bd	Bn	M	L	K
4	"	Bd	M	Bn	K	L
4	"	Bd	M	K	Bn	L
3	"	Bd	M	K	Bn	L
3	"	Bn	Bd	K	L	M
2	"	Bd	Bn	K	L	M
3	"	Bd	Bn	K	M	L
3	"	Bd	M	Bn	K	L
—	"	Bd	Bn	M	K	L
20	"	L	Bd	Bn	K	M
3	"	Bn	Bd	K	M	L

Experiments were made to establish the influence of the time element in the contact procedure. In some instances the duration of the contact was twenty-four hours. The organ pulps subject to this long exposure showed no greater action than those exposed for only one hour. There is good evidence that the action is normally complete at the end of the hour and that the greater part of the transformation occurs in the first few minutes. Organ pulps protected against putrefactive change show very little diminution in activity even after the lapse of several days.

The Infusion Method.—The second method employed in the study of the action of the different organs on phenol and indol consists of making intravenous infusions of solutions of these substances. Usually the injections were made until nervous symptoms appeared. The animals were then killed promptly and definite weights of the liver, kidney, muscle, brain and blood were subjected to distillation. Color tests were then made on the distillates, so that the quantity of phenol and indol found in the different tissues might be roughly compared. In order to minimize the influence of an admixture of blood with the cell pulps the animals were bled to death.

It is, of course, evident that owing to the time that must elapse between the death of the animals and the beginning of distillation, some transformation must go on during the interval, and thus give rise to a source of error in the results. The operations were, however, carried on with the smallest delay and it does not seem probable that this source of error can seriously influence the results.

A review of the results shows that there is much confusion in the order of the color tints. Nevertheless a certain degree of order can be observed in the gradation of color. The muscle occurs in the right hand column in ten of eighteen observations made with phenol and in three further observations the muscle is tied with the blood or liver. There is thus no doubt that the muscle distillates in these experiments exhibit less color than those of any other organ. The interpretation of this result is not entirely clear. It may be due to the fact that less phenol has passed into the muscles from the blood or it may be due to the fact that the phenol entering the muscles is more rapidly trans-

formed than the phenol which goes elsewhere. The results of the contact experiments are perhaps of help in the interpretation of the behavior of the muscle. It will be recalled that these observations showed the muscles to be less active than the liver or kidney. Unless, therefore, we assume that the activity of the muscles is relatively less outside the body than when the blood is passing through them, it appears probable that the muscle takes up less of the phenol than do other organs. According to this view the position of the muscle in the injection experiments would be largely due to their slighter absorption of phenol from the blood.

As regards the position of the kidney, liver, brain and blood in the phenol injection experiments, it is difficult to establish a definite order in the color results. The liver and kidney in the average of results appear not to differ much. Both show more color than the muscles. This cannot be attributed, in the light of the contact experiments, to an inferior ability to transform the phenol, but is due rather to the greater storage of phenol in these organs during the injection. Finally the brain and blood show a still stronger coloration than the liver and kidney. This is not surprising, as the contact experiments clearly indicate that the blood and brain are comparatively inactive. In our injection experiments sufficient time has not elapsed for the withdrawal of all the phenol from the blood.

The observations relating to the infusion of indol resemble those with phenol in the irregularity of the results. Certain facts nevertheless stand out clearly. In the case of the indol as with the phenol injection, the muscle is generally found to contain less of the infused substance at the end of the infusion than any other tissue. Here again some difficulty arises in interpreting the results. Is the small content of indol due to small absorption or to active tranformation into some other substance? Referring to the experiments with the indol contacts, it will be recalled that the muscle exerts less energy in transforming indol than either the liver or the kidney. Unless, therefore, the absorption of indol were less than in the case of the kidney and liver the position of the muscle in the series could not be accounted for. It seems probable that the slighter absorption together with the

moderately active transforming power of muscle suffices to explain its behavior.

Another fact which deserves comment is the position of the liver in the series. In a very large proportion of cases, the liver yielded more color than any other tissue. This striking result can hardly be accounted for except on the ground that the liver is more active than any other tissue in removing indol from the blood. There certainly is no reason to think the liver less active than other tissues, if we can be guided by our contact experiments. Our inference is that the liver takes indol from the blood much more actively than it does phenol. The ability of the liver to dispose of the indol stored up at the end of the infusion is illustrated by the fact that if the animal be not killed until the lapse of twenty or thirty minutes, the amount of indol stored in the liver is no greater than that found in the other organs. This fact has been verified by numerous observations.

The following observation has reference to the exceptional behavior of the tissues in an individual animal. As an example of individual variation on the part of the cells it appears of sufficient importance to be mentioned here. A rabbit weighing 1900 grammes received an infusion of saturated indol solution at the rate employed in other observations. Before the injection was begun the animal seemed unusually nervous. After the injection was begun, spasm came on early and the pupils became very small. The irritative symptoms soon wore away but the animal continued restless. The total amount of the infusion was 28 c. c. of a saturated solution in water. The animal died half a minute after the close of the injection, and was bled from the heart. The striking peculiarity in the behavior of this animal during the infusion was that the nervous symptoms—spasm, contracted pupil and restlessness—came on much earlier than is usual. Another peculiarity of the animal was the readiness with which the muscle substance could be torn.

On studying the organs in reference to their content of indol, it was found that the kidneys, the liver and the muscle contained only the smallest traces of indol. The blood and the brain on the other hand contained a large amount of indol, that is, they yielded a strong color reaction. This behavior on the part of the various tissues is quite without parallel in our experience. It is evident that in this case the liver, kidney and muscle were unable to remove indol from the blood with the

usual promptitude. The brain on the other hand, took out a larger amount than is usual and one is tempted to connect the very pronounced nervous symptoms observed in this animal with the relatively large quantity of indol found in the brain. It also seems likely that the inability of the liver, kidney and muscle to remove the poison from the blood was dependent on some constitutional peculiarity.

Nature of the Action Exerted by the Cells.—In the course of the preceding pages reference has repeatedly been made to the activity of the liver, kidney, muscles, etc., in effecting the transformation of phenol and indol. What is the nature of this transformation which leads to the disappearance of a portion of the phenol and indol brought into relation with the living cells? This question, which has a high degree of biological interest, cannot at present be satisfactorily answered. There are, however, certain facts relating to the subject which may be briefly considered here.

In the first place, it is clear, from the observations cited, that the processes by which phenol and indol are altered, are carried on much more actively in the cells of the liver and kidney, and probably also in those of the muscle, than in the blood. The property of effecting these changes is unquestionably inherent in the cells themselves and the transformation in the organism must occur in the blood in comparatively slight degree. This fact is in accord with the teaching of modern physiology in reference to the seat of the oxidizing changes that occur within the body during life.

In recent years several investigators have devoted attention to the nature of the oxidizing processes that are carried on in the cells. The methods and results of certain of these observers have some resemblance to the methods and results that have been referred to in this paper.

Jaquet * appears to have been the first to make observations as to the occurrence of oxidating processes in cells that have been removed from the body. He found that in lungs cut out of the body, one gramme of benzyl alcohol added to blood circulating experimentally through the organs was oxidized and yielded 185 grm. of benzoic acid. Jaquet found

* Ueber die Bedingungen der Oxydationsvorgänge in den Geweben, *Arch. f. exp. Path. u. Pharm.*, 1892, xxix, p. 386.

that the oxidation was as complete if normal salt solution, instead of blood, was employed as the circulating fluid. Moreover he found that the tissues of the horse (kidney and muscle) were active even after immersion in 80 per cent alcohol for 14 days, and that an extract of fresh, or alcohol-hardened, tissues in salt solution possessed good oxidizing powers. These, however, were lost by boiling.

Jamagiwa,* a pupil of Salkowski, soon confirmed the results of Jaquet. He found that different tissues were active in the following order of decrease: (1) spleen, (2) liver, (3) kidney, (4) pancreas, and (5) muscle. Then W. Spitzer † found that extirpated tissues in general have the power of inducing certain oxidative syntheses, for example, the synthesis of naphthol and paraphenyl diamine to form an indophenol.

In recent papers W. Spitzer † attributes the oxidizing action of the cell to its nucleoproteid. He finds that certain constituents of the proteid molecule, such as histon, also possess the oxidizing power, and reaches the conclusion that the presence of iron is essential to these activities.

Salkowski ‡ in his more recent work concludes that the ferment whose action is noted in the case of cells removed from the body cannot be identical with the oxidizing ferment of living cells because they do not oxidize certain substances which are readily converted in the living body. This is true, for example, of the oxidation of phenylpropionic acid to benzoic acid.

Such evidence as we now possess certainly indicates that the oxidative changes carried on by cells removed from the body have reference to substances that are readily oxidized. Thus the conversion of benzyl alcohol to benzoic acid, of salicylaldehyde to salicylic acid, of arsenious to arsenic acid, of benzol to phenol, of formic aldehyde to formic acid, and of methyl to formic acid, are all examples of comparatively readily induced oxidations. We are as yet unjustified in holding that such changes go on with equal vigor in cells outside the body, and in the fully living cells. Similarly, with reference to syntheses,

* Ueber das Oxydationsferment der Gewebe, *Centralbl. f. d. med. Wissenschaften*, 1894, xxxii, p. 913.

† Die zuckerzerstörende Kraft des Blutes und der Gewebe, Pflüger's *Archiv*, 1895, lx, p. 303. Die Bedeutung gewisser Nucleoproteide für die oxydative Leistung der Zelle, *Ibid.*, 1897, lxvii, p. 615.

‡ Zur Kenntniss des Oxydationsferments der Gewebe, Virchow's *Archiv*, 1897, cxlvii, p. 1.

there are certainly some which the extirpated cells cannot perform. Spitzer was unable to confirm the statement that the synthesis of urea from ammonium salts can be made to occur outside the body. Our work with indol indicates that the synthesis of indoxyl potassium sulphate cannot be accomplished by extirpated cells. It also seems improbable that the dead cells convert phenol into phenol-sulphuric acid. Yet we know that these syntheses are performed in the organism, probably by the very cells which fail to effect them outside the body. It is natural to ask whether the known oxidative activities of the dying cells, to which reference has already been made, are the same activities that lead to the disappearance of phenol and indol during contact experiments.

The order observed by Spitzer, Salkowski and others in the activity of the different cells outside the body agrees closely with the order observed in our contact experiments with phenol and indol. This fact suggested that the changes observed in the case of phenol are in the nature of an oxidative transformation. The oxidation of phenol leads to the formation of pyrocatechin and hydroquinone, substances which are met with in the urine under certain conditions of disordered metabolism. With a view to determining experimentally whether such a transformation goes on in the case of phenol when this is brought into contact with fresh cells, ten kilos of ox's liver were reduced to a fine pulp and brought into contact with a solution of phenol and subjected to the contact procedure which has already been described. After a sufficiently long period of contact an endeavor was made to isolate any pyrocatechin or hydroquinone which might have been formed during the period of contact. It was found impossible to recover either of these substances. With the method employed it would have been possible to recover these bodies had they been present in even smaller quantity than might be expected from the action of so large a bulk of liver cells upon phenol.* The negative results obtained indicate that the disappearance of phenol which occurs after contact with liver tissue does not depend upon a process of oxidation into dihydroxy-benzene. This conclusion is supported

* The method used by E. Baumann. See Hoppe-Seyler's Handbuch der Physiologisch- und Pathologisch-Chemischen Analyse, Berlin, 1893, p. 160.

by the fact that phenol introduced into the circulation leaves the body ordinarily as phenol sulphuric acid and not as hydroquinone or pyrocatechin.

In the case of indol it is thought that the first transformation that occurs in the organism is a process of oxidation into the indoxyl radical. It is possible that this oxidation occurs in the liver but this is by no means certain. If this be the case the action of the cells is perhaps comparable to the oxidative processes that have been studied by Spitzer and others. In what follows no further reference will be made to the fate of indol when acted upon by living cells. Attention will be confined to the discussion of the action of the cells upon phenol, an activity which apparently belongs in another category from that displayed toward indol.

The view held by Spitzer and by Salkowski that the oxidation which occurs through the action of cells is dependent on the presence of a ferment, raises the question whether such an agency will explain the behavior of cells toward phenol. The following facts bear directly upon this problem: Seven grammes of liver reduced to a pulp were brought into seventy-five cubic centimetres of absolute alcohol for one hour. The usual amount of phenol was then added to the liver pulp, and after a time subjected to distillation. The activity of the liver was slightly, but only slightly, impaired. In another experiment the usual amount of liver was boiled in fifty cc. of distilled water, and then brought into relation with phenol. Only the slightest falling off was noted in the action of the liver. In another case the liver was exposed to hot air, the temperature of the oven being gradually brought to 170° and kept there for twenty minutes. On exposure to the action of phenol, the liver showed no appreciable reduction in activity. Again, seven grammes of the liver were boiled with water, filtered hot, boiled again with water and filtered, and then boiled a third time and filtered. The pulp remaining on the filters showed itself capable of acting upon phenol with only a slight diminution in power. A test of the watery extract of the liver showed that it exerted only a very slight influence on phenol. Other trials made by bringing ten cc. of bichloride of mercury into relation with seven grammes of liver

showed that this procedure only slightly diminishes the activity of the cells.

Similar observations made with a 50 per cent solution of concentrated sulphuric acid yielded the same results. A 10 per cent solution of nitrate of silver was entirely without effect. In the case of the experiments with bichloride of mercury, sulphuric acid and nitrate of silver, the time of exposure of the liver cells was one hour.

In view of the facts just cited it appears in the highest degree improbable than any considerable ferment action can be attributed to the liver cells in connection with their behavior toward phenol. Another consideration which harmonizes with this conclusion is the fact that it is possible to completely exhaust the activity of the liver cells by repeated exposure to the action of phenol.

Although no wholly satisfactory explanation can be advanced as to the nature of the phenomenon under discussion, one is led to speculate whether we have not to deal with some loose combination of the phenol molecules with the molecules of the cell substance. That the combination between the cells and phenol is a loose one, is suggested by the fact that phenol absorbed from the intestine or injected into the circulation, reappears in the urine as phenol sulphuric acid.* It would also appear that the protoplasm of the cell suffers no damage when exposed to the action of phenol within physiological limits. The product formed by this combination of phenol with the cell substance may perhaps be likened to some of the numerous addition products known to chemistry.

The belief that the combination of phenol with the living protoplasm of the liver cells is a loose one, harmonizes well with the rapid elimination of phenol following the introduction of this substance into the circulation. It is obvious that it would be advantageous to the

* The phenol which enters the organism leaves the body almost quantitatively in the form of phenol, as is well shown by the following experiment: Into a large rabbit .080 grammes of phenol in watery solution were injected intravenously. The urine was collected for twenty-four hours and the phenol contained in the urine was determined by means of the iodine and sodium thiosulphate method. The quantity of phenol recovered was .0817 grms. It is possible that a few milligrammes of this phenol were derived from the intestine of the animal. The observation indicates, however, that the greater part of the phenol introduced was recovered.

organism for phenol to be temporarily held by the hepatic cells while the neutralization of the poison through combination with sulphuric acid is taking place. The manner in which such an arrangement would protect the nervous system, which is highly sensitive to the action of phenol, is sufficiently evident.

One other fact deserves mention in this connection. This has reference to the hypothesis advanced by O. Loew* that phenol acts upon living protoplasm by entering into combination with the labile groups which it contains, and, especially, groups possessing an aldehyde structure. With a view to testing this hypothesis the liver cells were subjected to the action of solutions of hydroxylamine, which enters readily into combination with aldehydes. Liver pulps treated in this way showed themselves to be as active or nearly as active in the conversion of phenol as normal livers.

In conclusion the opinion may be ventured that whatever may ultimately be learned of the nature of the phenomenon under discussion, it is likely that the capacity of cells to act upon phenol is only one expression of a function that can be exerted upon numerous allied aromatic substances, and it is even possible that a similar action may be exerted upon some non-aromatic bodies.

Action of Hepatic Cells under Pathological Conditions.—As already mentioned the degree of action exerted by the liver cells upon phenol and indol is fairly uniform. This fact suggested the possibility of recognizing alterations in the capacity of the liver cells to transform these substances under pathological conditions. It was, therefore, determined to make a series of studies of the liver cells of animals which had been subjected to different pathological influences. These observations include a study of the effects of prolonged anaesthesia, of poisoning by alcohol, ammonium chromate, morphine, etc., and of staphylococcus infection.

It is clear that it is a matter of considerable theoretical interest to know whether a definite impairment of functional activity in the cells of the liver and kidney is bound up with pathological influences like those just named. Efforts were, therefore, made to reach a decision

* Ein natürliches System der Gift-Wirkungen, München, 1893, p. 48.

upon this question although the difficulties attending these efforts were found to be somewhat discouraging.

The chief difficulty arises from the fact that the differences between the action of the normal and the pathological tissues are not great. This is what might have been expected in view of the resistance exerted by cells to the action of sulphuric acid, alcohol, etc. In order to establish the existence of such slight variations from the normal it was found necessary to exercise great caution in making comparisons between the distillates from the normal and the presumably pathological organs. It was soon found that the differences in the action of the kidneys and muscles were usually so slight in the pathological cases, as compared with the normal, that no inferences could usually be made. Only in the case of the liver—the organ in which action upon phenol and indol is the most energetic in conditions of health—was it possible to obtain satisfactorily results.

The distillates from the livers of pathological animals obtained through the "contact method" already described were compared not merely with individual distillates from the liver of normal animals, but also, in many instances, with mixtures of the distillates of the liver from many different animals. Great care was taken to make the conditions of the experiments the same as regards freshness of the organs, duration of the contact, quantity of distillates, amount of reagent added to give color, etc.

Anaesthesia by Ether and Chloroform.—The observations upon ether and chloroform included fifteen cases in which ether was used for anaesthesia and nine cases in which chloroform was employed. Of the fifteen cases of anaesthesia the liver cells were brought into contact with phenol in eleven, with indol in four. Of the nine cases of chloroform anaesthesia the livers were subjected to the action of phenol in four cases, to that of indol in five.

The rabbits subjected to etherization were kept under the influence of the anaesthetic for periods varying from five minutes to five hours in different cases. Although in four of the nine instances in which phenol contacts were made, the liver did not show the greatest activity of any member of the series, in the remaining cases the liver main-

tained its place as the most active organ. In most instances, however, the transformation of phenol was distinctly less than that effected by normal livers employed as controls. A similar difference was noted in the case of three of the four indol contact observations. In the fourth case where the animal had been under ether for two hours and a quarter the brain, blood, liver, kidney and muscle showed very slight differences in activity and the inference seems warranted that the activity of the liver, muscle and kidney are all below the normal.

The observations upon chloroform anaesthesia give even more definite results than those with ether. In two of the four cases where phenol was used (the animals being anaesthetized for three hours) the liver gave evidence of lessened activity. In the two remaining cases the liver, muscle and blood showed little or no difference in activity, a condition indicating that both liver and muscle were less active than normal. In four of the five observations with indol the liver was distinctly less active than the normal controls. In the fifth case the result was just the reverse.

Taking the results of these experiments with anaesthesia as a whole there can be no doubt that the prolonged action of chloroform and of ether depresses the activity of the liver in the conversion of phenol and indol. In exceptional instances it is not possible to detect this influence but the exceptions are so few that they do not affect the general result.

Poisoning by Alcohol.—Ten observations were made upon rabbits which had been subjected to the action of subcutaneous injections of 25 per cent alcohol. The periods of intoxication varied from a few hours to four days. The dose of alcohol was large in every instance and the animals were well under the influence of the poison. Thus in several cases the animal received 160 cc. of 25 per cent alcohol in three days and lay in a stupor most of the time.

Two of the ten observations were made with indol, eight with phenol. The periods of contact were varied from two to twenty hours in certain instances, the other conditions of the experiment being kept the same, but no differences in the results were detected which were referable to the differences in the length of contact. In a few cases

the activity of the liver was less than that of the normal controls, but more often no distinct falling off from the normal could be detected. Although we are not prepared to state that acute intoxication from alcohol is wholly devoid of effect upon the liver's action on phenol, our observations make it likely that there is either no influence of this kind or that it is very slight.

Poisoning by Ricin.—Thirteen trials were made to determine whether ricin poisoning exerts any influence on the ability of the liver to dispose of phenol and indol. It was thought probable that such an influence would be discernible as ricin has been shown to cause well-defined histological changes in the liver, kidney, etc.* The dose employed was unfortunately too large (.001 grm. to the kilo) and the duration of the poisoning was too short (less than thirty hours) to develop the degenerative lesions referred to. The results in the thirteen cases were negative.

Ammonium Chromate.—Observations were made upon four rabbits poisoned with ammonium chromate. The animals received two doses hypodermically of .025 ammonium chromate and were killed on the third day. The behavior of the liver cells did not positively differ from that of normal cells subjected to the same process of contact with phenol and indol previous to distillation.

Staphylococcus Infection.—The influence of staphylococcus infection was studied in two cases. The rabbits received five cc. each of a culture of *Staphylococcus pyogenes aureus* grown two days in bouillon. The organism was obtained from a patient with follicular tonsilitis. The animals were permitted to live one week, during which time they lost considerable weight. They were then killed by bleeding and the tissues subjected to the usual procedure. In both cases the differences in the activity of the brain, blood, muscle, kidney and liver were less than is usual in normal series. The distillate from the livers in the two cases was more colored than is usual in the case of normal livers.

These observations appear to indicate that in staphylococcus septi-

* Flexner, The Histological Changes Produced by Ricin and Abrin Intoxications, *Journal of Experimental Medicine*, 1897, ii, p. 197.

cæmia there is some impairment of the activity of the liver and kidney, but they can hardly be looked upon as conclusive.

Morphine Poisoning.—Eight observations were made upon rabbits under the influence of morphine. The animals received from five to eight grains of sulphate of morphine. Some of the animals lived only a few hours, others lived as long as two days. In four instances the activity of the liver as compared with that of normal controls was apparently somewhat lessened, but the deviation from the normal state was very slight. In every case except one the muscle showed greater capacity for transforming phenol than did the normal muscle. In several instances the difference was considerable as indicated by the color reaction. In several instances the kidney also showed an apparently increased activity.

Double Nephrectomy.—In five instances a double nephrectomy was performed with a view to seeing whether the removal of the kidneys is instrumental in impairing the activity of the liver. The animals were killed in from twenty-two to twenty-four hours. In all cases the liver showed a slight falling off in activity as compared with normal controls. The muscle and kidney, as in the case of morphine poisoning, showed in all cases somewhat greater capacity than usual but the differences were slight.

Infusion of One per cent Acetic acid Solution.—With a view to learning whether a reduction in the alkalinity of the blood has any influence upon the liver, a 1 per cent solution of acetic acid in a 1 per cent solution of sodium chloride was infused intravenously at the rate of 5 cc. a minute. The animal, a medium sized dog, went into coma before the completion of the infusion, which consisted of 130 cc. The animal excreted no urine during the period of injection. It was found that the activities of the liver, brain and muscle were about the same and distinctly inferior to the action of the kidney. A similar relationship has not been observed in normal dogs and it seems probable that the infusion of acid distinctly reduced the activity of the liver.

A similar observation with acetic acid, conducted on a large rabbit, gave a result comparable to that just noted in the case of the dog.

Benzoic Aldehyde Poisoning.—In these instances a saturated solution of benzoic aldehyde in water (1 part to 30) was infused into the venous circulation at the rate of 5 cc. per minute, until the animals died after developing symptoms of poisoning. The quantity of the solution infused varied from 80 to 210 cc. in the different cases. The livers from these rabbits showed some falling off in relation to their action on phenol. It was found, however, that the benzoic aldehyde employed in the experiments gave a slight reaction with Millon's reagent, and it is therefore possible, though not probable, that the abnormal behavior of the liver depends on the presence of phenol in the benzaldehyde in addition to the phenol added in the course of the contact procedure. It is to be regretted that this source of error exists, as it would be interesting to know whether the exhaustion of the oxidative activity of the liver upon a readily oxidizable substance like benzaldehyde necessarily causes impairment of the ability to transform or bind phenol.

Infusion of Urea.—Infusions of urea were made in several instances with a view to overworking the kidneys and observing whether such overwork causes any impairment in the activity of the kidney in converting phenol. The results obtained were wholly negative.

In addition to the various experimental observations recorded above a number of trials were made upon fatty and cirrhotic livers from human subjects. Owing to the delay incidental in obtaining human material after death it was impossible to obtain satisfactory results. It may be stated, however, that no marked deviations from the normal capacity of the liver to convert phenol were observed in organs the seat of the most advanced structural alterations.

It is probably safe to conclude that no pathological conditions which can be induced in the liver during life are capable of destroying, or even of greatly impairing, the activity of its cells in effecting the conversion of phenol. What is true of phenol in this connection is likely to hold good of indol. This view is supported by the few observations which have been made on indol.

The fact that this converting function of the liver is not greatly impaired in disease might perhaps have been predicted from what has

already been said of the remarkable functional resistance which the liver exhibits to the influence of injurious agencies outside the body. This preservation of function in disease is perhaps merely another expression of the fundamental biological properties which we have seen manifested by cells extirpated from the body. It is only reasonable to think that these properties are closely connected with the ability of the body cells to screen the organism and especially the nervous system from certain poisons. If this be so, the phenomena referred to in these pages deserve further study from pathologists.