STUDIES IN EDEMA.

VIII. THE INFLUENCE OF CAFFEINE ON ABSORPTION FROM THE PERITONEAL CAVITY AND THE INFLUENCE OF DIURESIS ON EDEMA.¹

BY MOYER S. FLEISHER AND LEO LOEB.

(From the Laboratory of Experimental Pathology of the University of Pennsylvania)

From the results of some earlier experiments (1, 2) it appears that the action of adrenalin in increasing the rate of absorption from the peritoneal cavity is due, not to the increased diuresis caused by this drug, but to an increase of the osmotic pressure of the blood. We have also shown in nephrectomized animals, and in animals upon which an operation had been performed, not directly affecting the kidneys, that changes in the osmotic pressure of the blood will account for the increased rate of absorption under such conditions. Furthermore, we have studied the influence of these various substances and conditions upon the distribution of sodium chloride and other osmotically active substances in the blood and peritoneal fluid.

We, therefore, determined to test the validity of our former conclusions by an investigation on the influence of diuretics on absorption from the peritoneal cavity. Through such experiments we hoped to learn definitely whether diuresis stands in direct relation to absorption from the peritoneal cavity. We injected 120 cubic centimeters of 0.85 per cent. sodium chloride solution into the peritoneal cavity of rabbits. Afterwards four injections of caffeine sodium benzoate were given, separated by periods of one-half hour; 0.25 gram caffeine were given with each injection. After two and a half hours the rabbits were killed and the fluid in the peritoneal cavity was collected. During the experiment the urine also was collected. We determined the freezing point and sodium

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chloride content of the blood serum, which was obtained through
defibrination and of the recovered peritoneal fluid, the sodium
chloride content of the urine, the refractive index of the blood
serum before and after the intraperitoneal injection of the sodium
chloride solution and the refractive index of the peritoneal fluid.

In other series of experiments we studied the influence of caffeine
on absorption in nephrectomized animals, in animals injected with
uranium nitrate, and in animals in which, instead of nephrectomy,
another operation, which did not directly affect the kidneys, had
been performed.

In one series of experiments we injected, instead of the sodium
chloride solution, distilled water into the peritoneal cavity of the
rabbits, and again determined the influence of caffeine on absorption.

In some of the experiments with normal animals, we injected the
caffeine subcutaneously, while in others the caffeine was injected
intraperitoneally; the effect of caffeine upon absorption was not
influenced by the method of administering the drug, as we recov-
ered approximately the same quantities of fluid from the peritoneal
cavity in each series of experiments. Therefore, in the following
reports we have made no distinction between experiments in which
caffeine was injected intraperitoneally and others in which it was
injected subcutaneously.

THE INFLUENCE OF CAFFEINE ON ABSORPTION IN NORMAL ANIMALS.

When caffeine was injected in addition to the sodium chloride
solution, we recovered from the peritoneal cavity an average of
82 cubic centimeters of fluid and collected during the experiments
an amount of urine averaging 67 cubic centimeters. Caffeine has
increased the diuresis considerably more than adrenalin, and not-
withstanding this increase in diuresis the amount of fluid absorbed
from the peritoneal cavity is slightly less than in experiments with
normal control animals which have received neither adrenalin nor
caffeine.

Both the relative and the absolute amount of sodium chloride
absorbed from the peritoneal cavity have been increased by the
injection of caffeine in normal animals. The blood serum of
animals injected with caffeine in addition to the sodium chloride
solution contains 0.46 per cent. of sodium chloride, while the serum of animals which had received an injection of sodium chloride solution but no caffeine contained 0.49 per cent. The urine secreted after the injection of caffeine contained 0.86 per cent. of sodium chloride, while that secreted by control animals which merely received an intraperitoneal injection of sodium chloride solution contained only 0.46 per cent. Thus under the influence of caffeine a much larger quantity of sodium chloride has been eliminated from the body through the kidneys and we find that in such animals the blood and recovered peritoneal fluid contain less sodium chloride than in control animals.

In spite of the lower sodium chloride content of the blood in experiments in which caffeine was injected, we find after the absorption of sodium chloride solution almost no difference between the freezing points of the blood in normal animals that had received an injection of caffeine and those that had not received such an injection. In the former the average freezing point was $-0.58^\circ$, in the latter it was $-0.586^\circ$. The average osmotic pressure of the recovered peritoneal fluids was also the same in both cases; the freezing point was $-0.59^\circ$ when caffeine was injected and $-0.593^\circ$ in control experiments. Since the osmotic pressure of both peritoneal fluid and of blood of normal animals injected with caffeine was almost the same as in control animals, while the sodium chloride content of both blood and peritoneal fluid was lower than in control animals, more osmotically active substances other than sodium chloride must have entered the blood and peritoneal fluid under the influence of caffeine.

If our explanation of the main factor determining the variations in absorption of fluid from the peritoneal cavity be correct, we should not find an increase of the osmotic pressure of the blood after an injection of caffeine, inasmuch as caffeine did not cause an increase in absorption from the peritoneal cavity. We, therefore, injected into normal animals at half-hour intervals the usual doses of caffeine without, however, injecting into such rabbits sodium chloride solution. These rabbits were killed shortly after the fourth injection of caffeine, and the average freezing point of the blood serum was found to be $-0.584^\circ$. In previous experi-
ments we have found the average freezing point of the blood of normal animals which had not been injected with either sodium chloride solution or caffeine to be $-0.6^\circ$. The injection of caffeine caused, therefore, a slight diminution in the osmotic pressure of the blood serum and this decrease corresponds to a slightly lessened absorption of fluid from the peritoneal cavity.

As a result of the absorption of fluid from the peritoneal cavity, the blood of animals, which in addition to the caffeine had received an injection of sodium chloride solution, showed an average decrease of the refractive index of the blood serum of only $0.00033$; in normal animals injected with sodium chloride solution without the addition of caffeine, the average decrease was $0.0133$. In experiments with caffeine the amount of water retained in the blood vessels as a result of the absorption of the sodium chloride solution has, therefore, been considerably less than in control experiments. This decrease in the retention of fluid was due to the increased diuresis, but, in addition, we have to consider the possibility that an increased amount of fluid may pass from the blood vessels into the tissues, in much the same manner as we have noted the passing of fluid out of the vessels into the tissues after an injection of adrenalin.

Thus we find that the injection of caffeine slightly lowers the osmotic pressure of the blood of normal animals, and probably as a result of this lowering of the osmotic pressure of the blood the absorption of fluid from the peritoneal cavity is found to be slightly diminished. The absorption of sodium chloride from the peritoneal cavity is, on the other hand, increased, notwithstanding the relative decrease of the sodium chloride in the blood. Since in experiments in which caffeine was injected, not only the sodium chloride percentage, but also the bulk of the blood after the absorption of sodium chloride solution is less than in control experiments without the injection of caffeine, the absolute quantity of sodium chloride retained in the blood must be diminished to an even greater degree than would appear from a consideration of the sodium chloride percentage alone. Both lessened retention of fluid in the blood vessels and the lower sodium chloride percentage of the blood may be due to two factors, namely, the increased elimi-
nation of both salt and water by the kidneys, and a specific action of caffeine which causes these substances to pass out of the blood vessels into tissues other than the kidney.

In these experiments as well as in experiments with normal animals in which no caffeine had been injected, we find both the osmotic pressure and the sodium chloride percentage in the recovered peritoneal fluid higher than in the blood serum.

**ABSORPTION IN ANIMALS INJECTED WITH DISTILLED WATER AND CAFFEINE.**

In rabbits which, in addition to an intraperitoneal injection of 120 cubic centimeters of distilled water, received repeated injections of caffeine, we recovered an average of 35 cubic centimeters of fluid from the peritoneal cavity, whereas in animals which received an injection of distilled water, but not caffeine, 44 cubic centimeters of fluid were recovered. Caffeine has, therefore, slightly increased the absorption of distilled water, since under the influence of caffeine 85 cubic centimeters of fluid were absorbed, while in experiments without injection of caffeine 76 cubic centimeters of fluid were absorbed. In the animals injected with caffeine, an average of 38 cubic centimeters of urine was secreted, while 14 cubic centimeters were secreted by animals which received no caffeine. In animals injected with caffeine the diuresis was not so markedly increased when distilled water was injected as when 0.85 per cent. sodium chloride solution was used.

In these experiments, as in experiments with normal animals injected with sodium chloride solution, caffeine has diminished the sodium chloride percentage of the recovered peritoneal fluid, but did not, as in experiments in which sodium chloride solution was injected, diminish the sodium chloride percentage of the blood serum. The diminution of the amount of sodium chloride which passed into the peritoneal cavity in the animals which, in addition to distilled water, were injected with caffeine, is due to the same factors which caused the sodium chloride content of the peritoneal fluid and blood to be less in animals injected with caffeine in addition to sodium chloride solution, namely, to the increased elimination of sodium chloride through the kidneys and to some influence
of caffeine causing sodium chloride to pass into the tissues of the body.

It appears that the addition of caffeine has not markedly influenced the osmotic pressure of the peritoneal fluid or the blood serum in animals injected with distilled water. Since the peritoneal fluid in animals injected with caffeine and distilled water contained a lower sodium chloride percentage than the peritoneal fluid in animals that did not receive caffeine, and yet the osmotic pressure of the fluids was the same, relatively more osmotically active substances other than sodium chloride must have passed into and been retained in the peritoneal fluid of animals which received injections of caffeine. More osmotically active substances other than sodium chloride have passed from the tissue into the blood in animals injected with caffeine in addition to distilled water than in animals in which caffeine was not injected. This follows from the fact that notwithstanding the increased amount of urine containing a large amount of osmotically active substances, and the large amount of osmotically active substances passing into the peritoneal fluid, the osmotic pressure of the blood has not decreased.

In experiments in which caffeine was injected in addition to distilled water, the osmotic pressure of the peritoneal fluid was less than that of the blood, in a similar manner as in experiments in which caffeine was not used.

The amount of fluid retained in the blood of rabbits which, in addition to distilled water, received injections of caffeine, was slightly less than the amount retained in the blood of animals not injected with caffeine, as shown by the diminution of the refractive index of the blood serum, which was 0.00125 with caffeine as against 0.00136 without caffeine.

We see that after an intraperitoneal injection of distilled water caffeine did not cause as marked a diminution in the amount of water retained in the blood as after an injection of 0.85 per cent. sodium chloride solution. This may be partly due to the fact that, in experiments in which caffeine was injected in addition to distilled water, the absorption of water from the peritoneal cavity was increased and that the elimination of fluid through the kidneys was under such conditions somewhat less than in experiments in which
516  Studies in Edema.

0.85 per cent sodium chloride solution was used instead of distilled water. Both of these factors tend to increase the amount of water retained in the blood. There may be another factor present, namely, the distilled water may perhaps injure or interfere with the mechanism through which the caffeine decreases the retention of fluid in the vessels.

**ABSORPTION IN NEPHRECTOMIZED ANIMALS INJECTED WITH CAFFEINE.**

In these experiments we studied the influence of caffeine on absorption in animals, in which twenty-four hours previously the vessels and ureters of both kidneys had been ligated. After the intraperitoneal injection of 0.85 per cent sodium chloride solution and the repeated injection of caffeine, in such animals we recovered an average of 28 cubic centimeters of fluid from the peritoneal cavity. Caffeine has distinctly increased the absorption of fluid in nephrectomized animals.

We have already shown that the osmotic pressure of the blood is an important, if not the most important, factor influencing the absorption of fluid from the peritoneal cavity. Could this increased absorption of fluid in nephrectomized animals be due to an influence of caffeine on the osmotic pressure of the blood? In order to determine the influence of caffeine on the osmotic pressure of the blood in nephrectomized animals, we injected caffeine into the peritoneal cavity of such animals every half hour, but omitted the injection of sodium chloride solution. In previous experiments we found the average freezing point of the blood serum of nephrectomized rabbits, which had not received caffeine, to be $-0.64^\circ$; in five nephrectomized animals which had received injections of caffeine, the average freezing point of the serum was $-0.70^\circ$.

We see, therefore, that the osmotic pressure of the blood is increased in nephrectomized animals which received caffeine; this increase represents the additive effect of nephrectomy and of the injection of caffeine. As we have seen, nephrectomy alone increases the osmotic pressure of the blood and the injection of caffeine in nephrectomized animals causes a marked additional rise of the osmotic pressure, while in normal animals caffeine does
not cause a rise in osmotic pressure. In normal animals the functioning of the kidneys seems to prevent the increase in the osmotic pressure of the blood under the influence of caffeine, while in nephrectomized animals the retention of substances which cannot be eliminated through the kidneys seems to cause an increase in the osmotic pressure of the blood.

Inasmuch as under the influence of caffeine the urine shows a relative decrease in the elimination of solids as compared with the elimination of fluid, this difference in the behavior of the osmotic pressure of the blood in normal and in nephrectomized animals under the influence of caffeine cannot alone be due to this increased elimination of fluid and solid substances from the blood into the urine in non-nephrectomized animals, since such an elimination in itself would rather tend to increase the osmotic pressure of the blood instead of diminishing it. If we assume that both fluids and solids pass directly from the blood into the urine, we have to assume that complicating factors come into play, in order to explain this difference in changes in the osmotic pressure of the blood in normal and in nephrectomized animals.

In agreement with our previous observations we again note a parallelism in the changes in the osmotic pressure of the blood and in the rate of absorption from the peritoneal cavity. In nephrectomized animals, caffeine increases the osmotic pressure of the blood and also increases the absorption of fluid, while in normal animals it has neither of these effects.

In nephrectomized animals the absorption of sodium chloride from the peritoneal cavity was increased by caffeine in the same way as in normal animals. We have seen that nephrectomy alone increases the absorption of sodium chloride from the peritoneal cavity and when caffeine is injected into such animals the absorption of sodium chloride is even more increased. In view of the lower sodium chloride percentage in the recovered peritoneal fluid and the greater amount of fluid absorbed in experiments with nephrectomized animals injected with caffeine, the absolute amount of sodium chloride absorbed from the peritoneal cavity is greater than in normal animals or in nephrectomized animals without the injection of caffeine. In normal animals which did not receive
caffeine, the absolute amount absorbed was 0.588 gram, in nephrectomized animals without caffeine it was 0.796 gram, and in nephrectomized animals with caffeine it was 0.857 gram.

Just as the sodium chloride percentage of the peritoneal fluid was lower in experiments with nephrectomized animals which received injections of caffeine, so the sodium chloride percentage of the blood serum was also lower than in nephrectomized animals which received no caffeine.

Since under the influence of caffeine the absorption of sodium chloride from the peritoneal cavity of nephrectomized animals is increased and the blood shows a lower sodium chloride percentage than in control experiments, more sodium chloride must have passed into the tissues than in experiments in which no caffeine was injected. We see, therefore, that when the kidneys are performing their normal function, caffeine causes an increased elimination of sodium chloride from the blood through the kidneys, and, if the kidneys do not functionate, caffeine still causes an increased amount of sodium chloride to pass out of the peritoneal fluid and blood. In the latter case it must be retained in the tissues generally or in a specific organ or tissue.

The average freezing point of the blood serum after the absorption of fluid from the peritoneal cavity was, in nephrectomized animals, --0.623° when caffeine was injected, and it was the same in nephrectomized animals without the injection of caffeine. The average freezing point of the peritoneal fluid in nephrectomized animals injected with caffeine was --0.623°, but without caffeine it was --0.59°. Thus we find that, in spite of the differences in the sodium chloride content of the blood in the two series of experiments, the osmotic pressure is the same in nephrectomized animals with and without the injection of caffeine, and more osmotically active substances other than sodium chloride must, therefore, be present in the serum of nephrectomized animals injected with caffeine than in the serum of nephrectomized animals without caffeine. In nephrectomized animals injected with caffeine the osmotic pressure of the recovered peritoneal fluid is higher, while the sodium chloride percentage is lower than in nephrectomized animals without caffeine. Thus the amount of other osmot-
ically active substances which have passed into the peritoneal cavity in nephrectomized animals is markedly increased under the influence of caffeine. It appears that caffeine causes the osmotically active substances other than sodium chloride to be absolutely and relatively increased in both the blood and peritoneal fluid and the surplus of these substances must have been drawn from the tissues.

The injection of caffeine into nephrectomized animals caused an average lowering of the refractive index of the blood of 0.0010 after the absorption of sodium chloride solution. Under the same condition in nephrectomized animals not injected with caffeine, the lowering of the refractive index amounted to 0.0015. Here and in normal animals caffeine diminished the retention of fluid in the blood vessels. Since in these experiments no fluid left the body through the kidneys, and yet a larger quantity of fluid was absorbed from the peritoneal cavity than in non-nephrectomized animals without the injection of caffeine, it appears that caffeine causes fluid as well as sodium chloride to leave the vessels and to enter the tissues. Adrenalin, on the other hand, accelerates, as we have found, only the movement of fluid and not of sodium chloride from the blood into the tissues. The fact that under the influence of caffeine the fluid content of the blood after the absorption of a sodium chloride solution from the peritoneal cavity is less than in control animals without the injection of caffeine proves that in nephrectomized as well as in normal animals the elimination of sodium chloride caused by the injection of caffeine is even considerably greater than would be indicated by the figures of the sodium chloride content of the blood.

**ABSORPTION UNDER THE INFLUENCE OF CAFFEINE IN OPERATED BUT NOT NEPHRECTOMIZED ANIMALS.**

In this series of experiments rabbits were operated on twenty-four hours previous to the study of the absorption. In each animal two lumbar incisions were made and the peritoneum was exposed; during the operation the peritoneum was repeatedly touched by the operator's fingers, care being taken that the peritoneum and the kidneys should not be injured.

When in addition to the sodium chloride solution we injected
caffeine intraperitoneally into such animals, we recovered an average of 41 cubic centimeters of peritoneal fluid at the termination of the experiment; while in operated animals which received no injection of caffeine, an average of 57 cubic centimeters of fluid was recovered from the peritoneal cavity, as stated previously. An average of 48 cubic centimeters of urine was secreted by the animals which received caffeine, while 18 cubic centimeters was secreted by the animals which received no caffeine. Here as in nephrectomized animals the injection of caffeine increased the absorption of fluid from the peritoneal cavity, but, as we have not determined the influence of caffeine on the osmotic pressure of the blood in these animals, we cannot positively state that in this case also an additive increase of the osmotic pressure of the blood due to the injection of caffeine is the cause of the increased absorption from the peritoneal cavity. We are, however, very much inclined to believe this to be the case, inasmuch as in animals operated in the manner described above as well as in nephrectomized animals, the osmotic pressure of the blood is raised and inasmuch as we have found that the injection of caffeine in nephrectomized animals causes an additional rise in the osmotic pressure. In view of the fact that absorption is not so markedly increased after the administration of caffeine in animals in which a lumbar incision has been made as in nephrectomized animals after the injection of caffeine, it is very likely that in the former animals caffeine does not cause so marked a rise of the osmotic pressure of the blood as it does in nephrectomized animals.

In animals in which a lumbar incision was made and in which caffeine was injected, the sodium chloride percentage of the recovered peritoneal fluid was 0.46 per cent. and the absolute amount of sodium chloride absorbed was 0.84 gram on the average. The average sodium chloride percentage in the blood serum was 0.44 per cent. The sodium chloride percentages in both peritoneal fluid and blood serum were, therefore, very small, while the quantity of sodium chloride absorbed from the peritoneal cavity was large. The influence of caffeine in operated animals has been such as to cause more sodium chloride to leave both the peritoneal fluid and the blood serum and to pass into the tissues. This observation is
in perfect accordance with what we have noted in normal and in nephrectomized animals regarding the influence of caffeine on the movements of sodium chloride.

The average freezing point of the recovered peritoneal fluid in animals with a simple lumbar incision which received caffeine was \(-0.575\)°, and that of the blood serum was \(-0.595\)°. This relation of the osmotic pressure of the peritoneal fluid to that of the blood serum was the same as in all other cases in which the osmotic pressure of the blood was raised above the normal level under the influence of experimental procedure. If we compare the results obtained in the case of operated animals which received caffeine with the results obtained in normal animals injected with caffeine, we note that after the absorption of fluid the osmotic pressure of the blood in operated animals injected with caffeine was somewhat higher than that of the blood of normal animals injected with caffeine; this is in conformity with the fact which we have pointed out in a previous paper that lumbar incision increases the osmotic pressure of the blood. While, therefore, the osmotic pressure of the blood in operated animals injected with caffeine was higher than that of the blood of normal animals injected with caffeine, the sodium chloride content was lower in operated animals; a relatively greater quantity of other osmotically active substances must, therefore, have passed from the tissues into the blood of these animals. Both the osmotic pressure and the sodium chloride content of the recovered peritoneal fluid of operated animals injected with caffeine was somewhat lower than that of normal animals which were injected with sodium chloride solution alone; the difference between the sodium chloride content, was, however, much greater than the difference between the osmotic pressure, so that in the peritoneal fluid of operated animals injected with caffeine, as well as in the blood serum of these animals, a large quantity of osmotically active substances other than sodium chloride was present. This is in agreement with the results noted in the other series of experiments in which caffeine was injected.

The decrease in the refractive index of the blood serum of operated animals injected with caffeine was \(0.00033\), while in operated animals which received no caffeine the average decrease
was 0.0015. Just as in normal animals, so in operated animals the amount of fluid retained in the blood vessels is diminished under the influence of caffeine. This is due to the increased diuresis caused by the administration of caffeine, but it may also be due in part to an increased output of fluid from the blood into the tissue in a similar way as we have observed an exchange of fluid in nephrectomized animals which received caffeine. We find, therefore, that in operated animals caffeine increases the absorption fluid from the peritoneal cavity and also the secretion of urine. It likewise increases the withdrawal of sodium chloride from both the peritoneal cavity and blood and causes a movement of other osmotically active substances into the blood and peritoneal cavity.

**INFLUENCE OF CAFFEINE ON ABSORPTION IN ANIMALS INJECTED WITH URANIUM NITRATE.**

In animals which received an injection of five milligrams of uranium nitrate three days before the absorption experiment, an average of 92 cubic centimeters of fluid were recovered from the peritoneal cavity after the intraperitoneal injection of sodium chloride solution and of caffeine. In experiments with animals injected with uranium nitrate which did not receive caffeine, 77 cubic centimeters of fluid were recovered, as we stated in a previous publication. The amount of urine is not increased by the injection of caffeine into such animals. Here, as in normal animals, caffeine has not only not increased but has somewhat decreased the rate of absorption from the peritoneal cavity.

In animals injected with uranium nitrate the freezing point of the blood serum after the absorption of fluid from the peritoneal cavity is slightly higher in experiments in which caffeine had been injected than in experiments in which no caffeine was injected. The average freezing point in the experiments with caffeine was 0.645°, and in experiments without caffeine, 0.63°.

The injection of caffeine in addition to sodium chloride solution into animals which had been injected with uranium nitrate causes an average reduction of the refractive index of the blood serum of 0.0012. In animals injected with uranium nitrate which did not receive caffeine the average reduction was 0.0022. In animals
which were injected with uranium nitrate three days previously caffeine causes, therefore, less fluid to be retained in the blood than in animals treated with uranium nitrate which received no caffeine. This may be partly due to the smaller amount of fluid which was absorbed after the administration of uranium nitrate, but caffeine again causes in all probability, even in uranium nitrate animals, fluid to pass out of the vessels and into the tissues.

We note, therefore, that in animals which had been injected with uranium nitrate three days previously caffeine has no accelerating effect on absorption in spite of the high osmotic pressure of the blood. The injection of uranium nitrate has brought into play some factor counteracting the influence of the high osmotic pressure of the blood on absorption from the peritoneal cavity; this we have already pointed out in our previous article on the influence of uranium nitrate. This factor may possibly consist of a change in the permeability of the endothelial cells of the blood vessels and peritoneum brought about under the influence of uranium nitrate.

**DISCUSSION OF RESULTS.**

Adrenalin and caffeine differ in some respects in their influence on absorption from the peritoneal cavity. Thus in normal animals adrenalin increases absorption, caffeine decreases it; in nephrectomized animals caffeine markedly increases absorption, but adrenalin increases it only slightly, if at all; in normal animals which were injected intraperitoneally with distilled water, adrenalin markedly increases absorption, caffeine increases very slightly the rate of absorption. In animals which have been injected with uranium nitrate three days previously adrenalin increases, while caffeine decreases the absorption of fluid.

In all series of experiments, the injection of caffeine causes less fluid to be retained in the blood vessels than in the corresponding control experiments. In some of these experiments the diminished retention of the fluid may be due to the increased diuresis, but certainly in nephrectomized animals and animals injected with uranium nitrate three days previously, there must exist some factor, which causes the fluid to pass from the blood vessels into the tissues. A similar movement of fluid from the blood into the tissues was noted in experiments in which adrenalin was injected.
In all series of experiments in which caffeine was injected the sodium chloride percentages of the recovered peritoneal fluid and of the blood serum were lower than in animals which had not received caffeine. Under the influence of caffeine the movement of sodium chloride follows, therefore, in the same direction which we found water to follow after administration of caffeine. Both salt and water pass from the blood into the tissues and the sodium chloride passes also from the peritoneal cavity into the tissues, either directly or indirectly.

The increased diuresis caused by caffeine is generally explained as being due primarily to an increased elimination of sodium chloride through the kidneys. Our experiments show that, under the influence of caffeine, sodium chloride passes out of the blood, even after the removal of the kidneys. When the kidneys are functioning, this salt is forced out of the body through the kidneys, but when the functions of the kidneys are entirely suppressed or when renal insufficiency exists, an increased quantity of sodium chloride passes out of the blood and is taken into the tissues under the influence of caffeine. The action of caffeine in increasing the sodium chloride elimination seems, therefore, not necessarily to be due to an action of caffeine upon the kidneys. The primary condition underlying the caffeine diuresis seems to be the elimination of sodium chloride from the blood, and the kidney appears to play only a secondary rôle in this process.

In contra-distinction to the relative diminution of sodium chloride in the blood and the peritoneal fluid under the influence of caffeine, other osmotically active substances in both blood and peritoneal fluid of animals injected with caffeine must be increased, since, as stated above, the osmotic pressure of these fluids is identical with that found in animals which did not receive caffeine. Large quantities of these other substances must have passed from the tissues into the blood. It may be that caffeine increases the metabolic activity and that as a result of this increase more osmotically active substances are formed in the tissues. This would not, however, explain the movement of sodium chloride into the tissues; there must be some specific condition which is unknown to us at the present time causing the passing of the latter substance from
the body fluids into the tissues. After the injection of adrenalin, on the other hand, a change in the movement of sodium chloride and of other osmotically active substances is usually absent, or if changes in the distribution of sodium chloride and other osmotically active substances are noted, they are not sufficiently regular and they do not permit us, therefore, to attribute to adrenalin a definite influence upon the distribution of these substances.

Several previous investigations have been reported concerning the influence of diuretic substances belonging to the caffeine groups on absorption. In all of these experiments the investigators merely tested this influence in either nephrectomized animals or in animals poisoned with uranium nitrate, and the conclusions at which they arrived are, therefore, at variance with the conclusions to be drawn from the results of our experiments.

Weber (3) studied the influence of theophyllin on the absorption of sodium chloride solutions from the subcutaneous tissue in nephrectomized animals. In control experiments he found that after an intravenous injection of theophyllin in nephrectomized animals in which no sodium chloride solution had been injected subcutaneously, the water as well as the chlorides and the ash in general were increased in the blood. When he injected into nephrectomized animals isotonic salt solutions subcutaneously and theophyllin intravenously, the water, ash and chloride contents of the blood were likewise increased; when he injected a hypertonic solution of sodium chloride subcutaneously the intravenous injection of theophyllin also increased the ash and chloride content of the blood, while, on the other hand, the blood became more concentrated than in control experiments in which theophyllin was not injected.

Weber found that under the influence of caffeine the sodium chloride content of the blood is increased, whereas we found the quantity of this salt in the blood to be diminished. This investigator found also that the fluid in the blood was increased when caffeine was injected, except in cases in which hypertonic solutions were used, while we found throughout our experiments that caffeine decreased the fluid content of the blood. On the other hand, our results coincide with Weber's, inasmuch as he found that the ash
of the blood was increased by the injection of caffeine and we have found that caffeine increases the amount of osmotically active substances other than sodium chloride in the blood.

One objection perhaps can be raised against Weber's experiments, inasmuch as he withdrew comparatively large quantities of blood from his animals in the beginning of the experiment. Furthermore, his experiments are not directly comparable with ours, inasmuch as he studied the absorption from the subcutaneous tissues, while we studied the absorption from the peritoneal cavity.

Georgopulos (4) injected ascitic fluid which he obtained from animals poisoned with uranium nitrate into the peritoneal cavity of nephrectomized rabbits and found that under the influence of caffeine the absorption of fluid was hastened.

From these experiments both of these observers drew the conclusion that diuretic substances of the caffeine group cause an increase in the rapidity of absorption, and in order to explain this phenomenon they introduced a so-called vital factor, assuming that these diuretic substances exerted a direct stimulating effect on the endothelial cells of the body and thus caused a more rapid movement of substances from the peritoneal cavity or tissues into the blood vessels. As we stated above, we explain the influence of caffeine on absorption in an entirely different manner and we believe that the previous investigators failed to note the influence of caffeine on the osmotic pressure of the blood in its relation to absorption because they did not carry out experiments concerning the influence of these substances on absorption in normal animals. This they probably omitted to do because they tacitly assumed that, inasmuch as in normal animals caffeine increases so very markedly the diuresis, it was a priori certain that the absorption would also be markedly increased under such conditions. Our experiments show, on the contrary, that the opposite effect is associated with the increased diuresis caused by caffeine in normal animals.

Both Georgopulos (4) and Richter (5) have found that repeated injections of caffeine or diuretics of a similar nature into animals poisoned with uranium nitrate diminished the degree of edema which was found at the end of the experiment. These observers believe, therefore, that these substances stimulate cells whose
function it was to absorb fluid from the tissues or from the peritoneal cavity. Their experiments were very complicated, inasmuch as they extended over several days and over various experimental periods, in which the conditions of the animals must have varied considerably, and if increased absorption actually took place under such conditions, it probably can be ascribed to an increase in the osmotic pressure of the blood which existed at some period of the experiment. Another possible explanation of their results, however, is the following: in their experiments the influence of caffeine in diminishing the edema was not a direct effect upon absorption, but their results were due to the fact that, in the earlier periods of their experiments, caffeine caused an increased elimination of the fluid which had been introduced into the stomachs of the animals. Inasmuch as the quantity of edematous fluid present in animals injected with uranium nitrate depends upon the amount of fluid available in the body, it necessarily follows that if considerable quantities are removed from the body through an increase in diuresis, less fluid becomes available for the formation of edema. In this case the influence of diuretics on the formation of edema is, therefore, an indirect one and is not due to a stimulating action which, according to the above named authors, diuretics of the caffeine group exert on absorption. According to our experiments, caffeine increases absorption only if it raises the osmotic pressure of the blood, but it may decrease edema by diminishing the fluid available for the formation of edema.

While we are thus able to explain the influence of caffeine and similar diuretics on absorption of water, the changes in the distribution of sodium chloride and of other osmotically active substances under the influence of these drugs, at the present time we can merely describe these changes without being able to state their cause. Hypothetically we might, however, suggest that certain metabolic processes which take place in the tissues under the influence of caffeine are mainly responsible for the changes in the distribution of sodium chloride and other osmotically active substances.

Throughout all our experiments in which we have studied the effect on absorption from the peritoneal cavity of various experimental conditions, such as the injection of adrenalin or caffeine,
Studies in Edema.

nephrectomy, or other operative interferences, and various combinations of these conditions, we found in all cases, in which the necessary determinations have been made, that the changes in the osmotic pressure of the blood induced by these experimental conditions were sufficient to explain the changes in the rate of absorption. Thus adrenalin increased the osmotic pressure of the blood and increased the absorption of fluid, while caffeine decreased the osmotic pressure of the blood in normal animals and diminished the rate of absorption. In nephrectomized animals both the osmotic pressure of the blood and the rate of absorption from the peritoneal cavity were increased, and in such animals caffeine caused an additive increase in the osmotic pressure of the blood and caused correspondingly a still greater amount of fluid to be absorbed from the peritoneal cavity. Not only nephrectomy but other operations also increased the osmotic pressure of the blood and thus increased the rate of absorption. In animals which were etherized we found no increase in the osmotic pressure of the blood and consequently the rate of absorption was unchanged.

That increased diuresis bears no causal relation to the increased absorption of fluid becomes evident if we consider the effect of caffeine on absorption in normal animals. In spite of the markedly increased amount of urine secreted, the amount of fluid absorbed from the peritoneal cavity is diminished in experiments with normal animals injected with caffeine. On the other hand, in normal animals which received an intravenous injection of adrenalin, the amount of urine secreted is the same as in control animals which received no adrenalin, but, nevertheless, the absorption of fluid is increased.

At present we do not wish to state that the osmotic pressure of the blood is the only factor determining the rate of absorption of fluid from the peritoneal cavity. We have pointed out certain conditions in which complicating factors arise, as in animals injected with uranium nitrate and in animals affected with peritonitis. Leaving out of consideration these and other cases of a complicated character, we may state that in our experiments changes in the osmotic pressure of the blood are sufficient to explain the principal variations in the rate of absorption which we have noticed.
We add here two tables, in the first of which we show the relations existing between the osmotic pressure of the blood, the diuresis and the absorption of fluid from the peritoneal cavity; in the second we give the averages of the results obtained in all our experiments concerning absorption from the peritoneal cavity.

**TABLE I.**

Relations between the Osmotic Pressure of the Blood, Diuresis and Absorption of Fluid from the Peritoneal Cavity. (Compared with the Results Obtained in Normal Animals Injected with 0.85 per cent. Sodium Chloride Solution.)

<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>Osmotic pressure of blood</th>
<th>Diuresis</th>
<th>Absorption of fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection of adrenalin</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Injection of caffeine</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Nephrectomy</td>
<td>+</td>
<td>O</td>
<td>+</td>
</tr>
<tr>
<td>Various operations</td>
<td>+</td>
<td>slight + or =</td>
<td>+</td>
</tr>
<tr>
<td>Etherization</td>
<td>=</td>
<td>=</td>
<td>=</td>
</tr>
<tr>
<td>Nephrectomy and caffeine</td>
<td>++</td>
<td>O</td>
<td>++</td>
</tr>
<tr>
<td>Injection of uranium nitrate one day previously</td>
<td>+</td>
<td>+</td>
<td>=</td>
</tr>
<tr>
<td>Injection of uranium nitrate three days previously</td>
<td>+</td>
<td>-</td>
<td>=</td>
</tr>
<tr>
<td>Peritonitis</td>
<td>+</td>
<td>= or -</td>
<td>-</td>
</tr>
</tbody>
</table>

(= increased; = decreased; = unchanged; O absent; ++ markedly increased.)

Certain phenomena which we observed in our experiments we cannot explain at the present time. For instance, in animals injected with uranium nitrate the osmotic pressure of the blood is increased, but the rate of absorption of fluid from the peritoneal cavity is not increased. The cause of the changes in the distribution of sodium chloride or other osmotically active substances in nephrectomized animals, in animals poisoned with uranium nitrate, or in animals injected with caffeine, is also at present obscure. Before we turn for an explanation of these phenomena to the assumption of the so-called vital activity of the endothelial cells of the peritoneum or of the blood vessels, we prefer to wait until the analysis of the changes occurring under the various experimental conditions which we produced in our investigations shall have been carried much farther, and, for the present, to suspend judgment.
### Studies in Edema

#### TABLE II.
**Average Results of Experiments on Absorption from the Peritoneal Cavity.**

<table>
<thead>
<tr>
<th>Blood before experiment</th>
<th>Recovered fluid</th>
<th>Serum</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blood before experiment</td>
<td>Recovered fluid</td>
<td>Serum</td>
</tr>
<tr>
<td>Normal; 0.85 per cent. sodium chloride</td>
<td>455</td>
<td>0.06</td>
<td>77.53</td>
</tr>
<tr>
<td>Normal; 0.85 per cent. sodium chloride; adrenalin intraperitoneally</td>
<td>—</td>
<td>—</td>
<td>41.52</td>
</tr>
<tr>
<td>Normal; 0.85 per cent. sodium chloride; adrenalin intravenously</td>
<td>455</td>
<td>0.06</td>
<td>62.38</td>
</tr>
<tr>
<td>Normal; distilled water</td>
<td>—</td>
<td>—</td>
<td>44.40</td>
</tr>
<tr>
<td>Normal; distilled water; adrenalin intraperitoneally</td>
<td>—</td>
<td>—</td>
<td>21.36</td>
</tr>
<tr>
<td>Normal; 1.5 per cent. sodium chloride</td>
<td>—</td>
<td>—</td>
<td>116.59</td>
</tr>
<tr>
<td>Normal; 1.5 per cent. sodium chloride; adrenalin intraperitoneally</td>
<td>—</td>
<td>—</td>
<td>81.62</td>
</tr>
<tr>
<td>Nephrectomy</td>
<td>466</td>
<td>0.64</td>
<td>47.05</td>
</tr>
<tr>
<td>Nephrectomy + adrenalin</td>
<td>—</td>
<td>—</td>
<td>43.49</td>
</tr>
<tr>
<td>1 day uranium nitrate</td>
<td>495</td>
<td>0.615</td>
<td>74.585</td>
</tr>
<tr>
<td>1 day uranium nitrate + adrenalin</td>
<td>—</td>
<td>—</td>
<td>46.50</td>
</tr>
<tr>
<td>3 day uranium nitrate</td>
<td>466</td>
<td>0.675</td>
<td>77.505</td>
</tr>
<tr>
<td>3 day uranium nitrate + adrenalin</td>
<td>—</td>
<td>—</td>
<td>64.57</td>
</tr>
<tr>
<td>Etherization</td>
<td>0.59</td>
<td>0</td>
<td>70.57</td>
</tr>
<tr>
<td>Lupol's solution</td>
<td>—</td>
<td>—</td>
<td>71</td>
</tr>
<tr>
<td>Lumbar incision</td>
<td>—</td>
<td>—</td>
<td>6.24</td>
</tr>
<tr>
<td>Abdominal incision</td>
<td>—</td>
<td>—</td>
<td>0.603</td>
</tr>
<tr>
<td>Thoracic incision</td>
<td>—</td>
<td>—</td>
<td>0.605</td>
</tr>
<tr>
<td>Peritonitis</td>
<td>—</td>
<td>—</td>
<td>13.3</td>
</tr>
<tr>
<td>Normal; caffeine</td>
<td>0.58</td>
<td>2</td>
<td>82.47</td>
</tr>
<tr>
<td>Distilled water and caffeine</td>
<td>—</td>
<td>—</td>
<td>35.32</td>
</tr>
<tr>
<td>Nephrectomy and caffeine</td>
<td>—</td>
<td>—</td>
<td>0.70</td>
</tr>
<tr>
<td>Lumbar incision and caffeine</td>
<td>—</td>
<td>—</td>
<td>41.46</td>
</tr>
<tr>
<td>3 day uranium nitrate and caffeine</td>
<td>—</td>
<td>—</td>
<td>92</td>
</tr>
</tbody>
</table>

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**Notes:**
- All values in milligrams per centimeter of fluid.
- Dilution values are in milliliters per centimeter of fluid.
- All experiments conducted under sterile conditions.
CONCLUSIONS.\textsuperscript{2}

I. In normal animals the injection of caffeine slightly diminishes the absorption of fluid from the peritoneal cavity, in spite of the fact that the amount of fluid and sodium chloride eliminated through the kidneys is markedly increased. The lessened absorption of fluid is due to a slight lowering of the osmotic pressure of the blood.

II. In nephrectomized animals caffeine increases the absorption of fluid from the peritoneal cavity; the increase in absorption is greater in nephrectomized animals which received caffeine than in nephrectomized animals which did not receive this substance, and it is due to additive increase in the osmotic pressure of the blood. In a similar manner, caffeine increases the absorption of fluid from the peritoneal cavity in animals in which, instead of nephrectomy, other operations, not directly affecting the kidneys, had been performed. In this case also the increase in absorption is presumably preceded by and due to an increase in the osmotic pressure of the blood.

III. In animals injected with uranium nitrate three days previously, caffeine diminishes the absorption of fluid from the peritoneal cavity, notwithstanding the high osmotic pressure of the blood which we observe in such animals. This agrees with the results of our previous experiments in which we found that in animals injected with uranium nitrate the absorption of fluid is not increased in spite of the rise of the osmotic pressure of the blood.

IV. At the time of the conclusion of the absorption experiments, the amount of fluid retained in the vessels was found to be diminished in each series in which caffeine was used. Only in certain cases can this be due to the increased amount of fluid leaving the blood vessels through the kidneys; in other cases it can only be due to a movement of water from the blood vessels into the tissues caused by the injection of caffeine.

V. In normal animals, in nephrectomized animals and in animals in which an operation not directly affecting the kidneys had been performed, caffeine causes an absolute and relative increase in the

\textsuperscript{2}In the series of experiments reported in this communication, 10 rabbits were used in a corresponding number of individual experiments. The protocols of the individual experiments have been omitted.
elimination of sodium chloride from the peritoneal fluid, as a result of which the remaining peritoneal fluid shows a lessened content of sodium chloride. Caffeine causes also a decrease in the sodium chloride content of the blood. We see, therefore, that under the influence of caffeine a greater amount of sodium chloride is eliminated from the body fluids into the tissues or through the kidneys. The factors which cause the sodium chloride to leave the body fluids are probably primarily responsible for the diuresis which takes place after administration of caffeine. In the case of caffeine and other similar substances the diuresis is, therefore, in all probability not due primarily to a specific action of the kidney, but to conditions which affect the distribution of sodium chloride in the body.

VI. The distribution coefficient of other osmotically active substances differs from that of sodium chloride. These other substances have a tendency to move into the body fluids in increased quantities under the influence of caffeine.

VII. Summarizing all experiments in which we studied the absorption from the peritoneal cavity, we may state that changes in the osmotic pressure of the blood represent the principal factor in explaining the variations in the rate of absorption of fluid from the peritoneal cavity.

VIII. There exists no direct relation between an increase in the rate of absorption of fluid from the peritoneal cavity and an increase in the amount of urine secreted. If it should be found that even at a period following the injection of caffeine, later than that at which we have studied the absorption, a rise of the osmotic pressure of the blood does not appear, then we may state that the diminution in the amount of edema in the body cavities resulting from the administration of caffeine is entirely due to an inhibition of the production of edema and not to an increased absorption of fluid from the serous body cavities.

BIBLIOGRAPHY.