On the Action of Paraphenylenediamine.

By

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The discovery by Dubois and Vignon of the fact that extreme acute oedema is caused by injecting paraphenylenediamine under the cutis of a dog has been confirmed by many subsequent workers in such animals as the rabbit and the cat. Yet arguments do not agree as to whether this oedema is general or localized; while we meet with a few arguments concerning the cause of oedema, even these disagree, Meissner¹⁾ claiming that it is connected with the parasympathetic nerves, Gibbs²⁾ that it is caused by some unknown alteration of the blood, while Tainter and Hanzlik³⁾ content that it is due to an increase in the permeability of blood vessels. With the hope that it might be possible to contribute something towards the solution of this problem, I have carried out the following experiments on rabbits, using paraphenylenediamine in the form of the hydrochloride.

I. LOCALIZATION OF THE OEDEMA.

It has been thought that the oedema of paraphenylenediamine appears strictly localized in the regions of the head and neck. But Gibbs, who found the formation of oedema not only in the regions mentioned, but also in the dorsum of the paws, the tail-base and the flanks of cats poisoned by paraphenylenediamine, considers it to be a general one extending over many tissues, only being more pronounced in the head and neck than elsewhere, the same as in the case of oedema produced by the perfusion of an animal with Ringer's solution. This view, however, is contradicted by Tainter and Hanzlik, who experimenting on rabbits failed to reveal oedema in regions of the body other than the head and neck.

In my experiment on rabbits injected with paraphenylenediamine under the cutis of a flank, in a dose of 0.2 grm. per kilo there was always found some sign of oedema at the site where the injection was made. In other parts of the body than the injected site and the head and neck no oedematous swelling could be observed. But when animals receiving the same dose of the poison were infused with Ringer's solution at a rate of approximately 1 c.c. per kilo per minute from a posterior auricular vein for hours, a very distinct oedema spreading all over the subcutaneous tissue of the injected side of the body was invariably observed. The armpit and the groin of the same side were also affected. A detailed protocol is given herewith.

Rabbit, male, 1.7 kilos.

- 3 : 30 0.34 grm. of paraphenylenediamine dissolved in 15 c.c. of water is injected under the cutis of the right flank.
- 4 : 30 Infusion of Ringer's solution is commenced.
- 5 : 00 Quantity of the fluid infused reaches 50 c.c.; the tongue expands, the tip reaching the incisor; the animal becomes uneasy and makes spasmodic movements; tracheotomy is performed.
- 5 : 30 Quantity of the fluid infused reaches 100 c.c.; respiration becomes frequent, uneasiness increases; oedema develops on the lips and between the lower jaws.
- 6 : 00 Quantity of the fluid infused reaches 160 c.c.; the face and neck are swollen, the tongue protrudes; wound secret increases markedly.
- 7 : 00 Quantity of the fluid infused reaches 253 c.c.; the animal shakes its legs continually and is extremely uneasy; no emiction occurs. The animal is killed.

Dissection reveals the formation of oedema not only in the head and neck, but also in the subcutaneous tissue of the right side of the body. The oedematous swelling in the latter region highly develops at the injected part, being measured approximately 1 cm. in thickness, and gradually decreases with the distance from there. The right armpit is slightly oedematous, but the right groin is much more swollen. The appearance of the oedematous tissues in the body is similar to that in the head and neck.

The marked oedema spreading over the subcutaneous tissue of the injected side observed in this experiment cannot be referred to hydraemia caused by the infusion with Ringer's solution, for the infusion made in the same manner was proved not to cause perceptible oedema, and even when this was caused by the infusion with a larger quantity of the fluid it never localized in one side of the body, but to paraphenylenediamine having an action to produce oedema in other parts than the head and neck, which is to be supported by the hydraemia. It can therefore be said that paraphenylenediamine has a property to cause oedema in many tissues of the body, there being however difference in the intensity of these and thus the oedema appearing specially pronounced in the regions of the head and neck. This seems to be sufficient to confirm the view advanced by Gibbs.

II. INFLUENCE OF DRUGS UPON THE OEDEMA.

It is still a question whether the oedema produced by paraphenylenediamine can be prevented by certain drugs, such as calcium salt, atropine etc., and the determination of this problem seems to be desirable for the assay of the cause of the oedema.

1. Calcium chloride.

Meissner states that in rabbits the production of paraphenylenediamine oedema was entirely prevented by subcutaneous injection of a large quantity of calcium chloride. But this is not confirmed by subsequent workers, Tainter and Hanzlik, who, experimenting on the same species of animal, found that administration of calcium chloride could only delay the onset of oedema, without preventing its production. If calcium chloride were capable of retarding the oedema production, it might possibly prevent the production, if a sufficient dose of it were employed. This is the reason why I have performed the following experiment.

Rabbits weighing about 2 kilos were injected with calcium chloride in a dose of 10 c.c. of a 10 per cent solution five times at thirty minutes intervals under the cutis of one of the flanks, as was the case with Meissner's experiments, and thirty minutes after the last injection paraphenylenediamine in a dose of 0.2 grm. per kilo, which is sufficient to produce a marked oedema in an untreated animal, was introduced under the cutis of the other flank. In this experiment no sign of oedema either on the head or on the neck was observed. On autopsy also there was found no trace of oedema on these parts, but a distinct oedema was seen in the parts injected with calcium chloride.

It is therefore certain that the subcutaneous injection of calcium chloride prevents the formation of paraphenylenediamine oedema, as Meissner states, but it is very doubtful whether this can be attributed to the action of calcium chloride in decreasing the permeability of the blood vessels, as he thinks, or not. The constant appearance of marked oedema around the site injected with the salt solution seems to indicate rather that the hypertonic solution of the salt, which, is slowly absorbable, will cause withdrawal of water from the blood, and thus promote the production of oedema around the injected sites, while inhibiting it on the head and neck. To determine whether this is true or not it is desirable to perform an experiment with an intravenous injection of the salt, which is however difficult to practise because the quantity large enough to produce severe circulatory disturbance will make ambiguous the result of the experiment. Therefore I carried

out a similar experiment using sodium chloride instead of the calcium salt, in order to investigate whether the former also has the same action upon the production of oedema.

2. Sodium chloride.

Rabbits weighing about 2 kilos were injected with 10 c.c. of 10 per cent sodium chloride solution five times at intervals of thirty minutes under the cutis of one flank and thirty minutes after the last injection with 0.2 grm. per kilo of paraphenylenediamine under that of the other.

In this experiment no perceptible oedema could be detected in the regions of the head and neck in the course of four hours, except some swelling of the tongue in three cases out of five. On the other hand, pronounced oedema was always seen, on autopsy, round the sites injected with sodium chloride solution.

Thus, there can be found no essential difference in the action upon the oedema of paraphenylenediamine between sodium chloride and calcium chloride. Hence the prevention of oedema by the latter can not be attributed to its specific action on the permeability of the blood vessels, but to the withdrawal of water from the blood by osmotic action.

3. Atropine.

Meissner states also that the oedema after paraphenylenediamine in rabbits was entirely prevented by the injection of atropine. But this does not agree with the result obtained by Gibbs who made experiments on cats, and also with that obtained by Tainter and Hanzlik who experimented on rabbits.

In my experiment performed on three rabbits receiving 0.2 per kilo of paraphenylenediamine under the cutis, subcutaneous injection of atropine in doses of 0.01 per kilo at intervals of thirty minutes also failed to show any preventive action, oedema developing in the head and neck and death following as usual. There exists therefore no relation between the parasympathetic nerves and the formation of paraphenylenediamine oedema.

III. INFLUENCE ON THE CIRCULATION.

Gibbs presumes that paraphenylenediamine is not a capillary poison, as he could not find any fall of blood pressure due to the poison until a typical oedema appeared. According to Hanzlik, a small dose injected intravenously caused a rise of blood pressure with acceleration and systolic tendency of the heart, while a large dose caused a fall with cardiac depression. There is therefore no doubt that the poison has an action to pro-

duce a certain change in the blood pressure. But the point of interest to me is the question as to what sort of change occurs in the circulation previous to the formation of oedema and at its height through sufficient quantity of the poison to bring about a marked oedema. Yet this has not been studied.

Rabbits of about 2 kilos weight were anaesthetized with urethane, as this had been proved not to prevent the formation of oedema due to paraphenylenediamine, though somewhat to delay its onset, and tracheotomized in order to avoid asphyxia due to swelling of the vocal cords. Blood pressure was measured by means of a Hg-manometer connected to a femoral artery instead of a carotid, for ligature of the latter has been stated to prevent the production of paraphenylenediamine oedema. About twenty minutes after subcutaneous injection of the poison in a dose of 0.2 grm. per kilo the blood pressure used to begin to rise gradually and in about thirty minutes after this reached its maximum and continued for some time after the oedema reached its height. The rise in blood pressure was, however, not marked, only reaching about 10 mm. The rate of pulse slightly decreased with the rise of blood pressure. When the same dose of the poison was given slowly into a posterior auricular vein, similar changes were caused, except that in this case the rise in blood pressure was always preceded by a slight fall.

In experiments, in which contraction of the heart was recorded by Cushny's myocardiograph and volume of the small intestine by a plethysmograph, it was found that after injection of the poison amplitude of the heart and volume of the intestine increased in accordance with the rise of blood pressure. And in other experiments performed on the excised auricle and intestine perfused with Ringer's solution containing acacia, injection of the poison solution in effective concentration into the inflow tube was followed by a diminution of the outflow fluid, indicating contraction of the blood vessels.

The rise in blood pressure after paraphenylenediamine is therefore due to an increase in the volume of blood expelled from the heart and the increase in the volume of the intestine seems to be a passive change caused by the rise of blood pressure. This change in blood pressure would of course play no important rôle in the production of oedema, but it may participate in accelerating it.

In this connection I made experiments in order to know whether oedema of an excised auricle due to perfusion of Ringer's solution would be influenced by adding paraphenylenediamine to the perfusion fluid. One auricle excised from a rabbit was perfused with Ringer's solution contain-

ing acacia and the other with the same solution containing also paraphenylenediamine, and the intensity of resulting oedema was compared by their weight. In this experiment it was observed that the formation of oedema was greatly delayed by the poison, even when it was perfused with a solution of a concentration not sufficient to cause contraction of the vessels. A similar result was obtained in an experiment on the tongue of a rabbit perfused from the carotid arteries, ligating all the blood vessels except the lingual arterics.

This result agrees with that obtained by Tainter and Hanzlik in an experiment on the head and the leg, and seems to indicate that the poison has no action to increase vascular permeability and that the oedema production must be explained in some other way.

IV. INFLUENCE ON THE LYMPHATIC FLOW.

Gibbs attempted to examine the action of paraphenylenediamine on the flow of lymph from a cannula inserted in a jugular vein where the lymphatic ducts from the head join together and found it to be very difficult to make continuous observation because of clotting of the lymph after injecting the poison, so that only one case succeeded. In this case he found that after a temporary increase in the flow, a decrease continued for a long time and another increase occurred just prior to death, and he assumed that this blockade of lymph is not an important factor in the formation of oedema. This is true, but the change in the lymphatic flow seems to deserve careful attention in interpreting the mechanism of the oedema production. Accordingly the following experiments were carried out in order to investigate the relation between the change in the lymphatic flow and the progress of the oedema. Care was taken in this case to guard as much as possible against loss of lymph.

A rabbit anaesthetized with urethane was fastened down on its back and a large incision was made along the median line of the neck and a sufficient space opened by separating the *m. sternomastoideus* and *m. sterno-hyoideus*. When the latter muscle was drawn slightly towards the opposite side, a fairly large lymph duct was found on the outer side running somewhat parallel to the carotid artery. After having separated that duct from its surroundings for a length of about 2.5 cm. a ligature was made near the base of the neck and a piece of paraffin paper was placed between. When an incision was made at the peripheral part in the duct lymph flowed out and continued without any stoppage due to clotting during the time ne-

cessary for the experiment. A piece of filter paper of about 2×1.5 cm. previously weighed was held to the opening of the duct so as to absorb lymph for a certain time and the quantity of lymph absorbed was estimated by weighing. Working on rabbits of about 2 kilos in this manner, the quantity of lymph flowing out in ten minutes was found to differ individually, ranging from 0.022 to 0.044 grm., but in the same animal the rate of the flow was almost constant for four or five hours though it showed a tendency slowly to decrease.

TABLE I.

Effect of paraphenylenediamine on lymphatic flow.
(The quantity of lymph per 10 minutes in grm.)

No. of experiment	1	2	3	4	5	6	7
Body weight (kilos)	2.0	1.8	2.1	2.3	2.0	1.9	2.0
0'- 10'	0.038	0.030	0.038	0.044	0.036	0.022	0.040
- 20	0.039	0.031	0.036	0.042	0.036	0.022	0.039
- 30	0.040	0.028	0.038	0.038	0.034	0.020	0.040
- 40	0.040	0.028	0.036	0.038	0.035	0.020	0.040
- 50	0.038 ↓	0.028 ↓	0.038 ↓	0.042 ↓	0.038 ↓	0.020 ↓	0.040 ↓
- 60	0.036	0.027	0.037	0.040	0.032	0.021	0.031
- 70	0.040	0.026	0.038	0.037	0.030	0.022	0.038
- 80	0.038	0.027	0.037	0.036	0.034	0.020	0.038
- 90	0.036	0.026	0.037	0.038	0.032	0.020	0.039
-100	0.032	0.025	0.035	0.037	0.030	0.020	0.038
-110	0.022	0.027	0.032	0.036	0.034	0.022	0.034
-120	0.020	0.025	0.026	0.035	0.032		0.036
-130	0.018+	0.022	0.014	0.030	0.028	0.020	0.032
-140	0.016	0.020	0.018	0.026	0.026	0.020	0.035
-150	0.020	0.020+	0.030+	0.020	0.020+	0.018	0.030
-160	0.024	0.018	0.038	0.022	0.018	0.015	0.026
-170	0.030	0.022		0.033+	0.022	0.010+	0.022+
-180	0.042++	0.024	0.052	0.039	0.028	0.010	0.020
-190	0.056	0.030	0.040++	0.050	0.034++	0.008	0.024
-200	0.062	0.033		0.062++	0.039	0.010	0.033
-210	0.070+++	0.040++		0.080	0.050	0.012	0.048++
-220	0.080			0.080	0.058	0.013	0.058
-230	0.108			0.090+++	0.070+++	0.018++	0.072
-240				0.095	0.084	0.020	0.090
-250				0.110	0.100	0.020	0.108+++
-260						0.024	0.135
- 70						0.032	0.168
-280						0.035	
-290						0.038+++	
-300						0.050	

Rabbits anesthetized with urethane and tracheotomized. At ↓ 0.2 grm. paraphenylenediamine per kilo was injected subcutaneously.

+ indicates the first appearance of oedema in the tongue, ++ protrusion of the tongue and of the eyeballs and +++ marked oedema.

In this experiment, subcutaneous injection with 0.2 per kilo of the poison was followed by the usual effect, the oedema appearing in one and a half to two and a quarter hours, approaching its maximum about thirty minutes after that and death occurring about five hours after the injection. The rate of lymph flow commenced to decrease almost simultaneously with the appearance of the oedema and continued until this developed markedly, but after some time it turned to increase, and when this approached its maximum it exceeded that previous to the injection, as is shown in Table I.

Thus there is no doubt that paraphenylenediamine causes an increase of the lymph flow by accelerating its formation, and the preliminary decrease in the flow can be explained only by accepting an increased imbibition of water by the tissues. The cause must therefore be due to this increased imbibition and the increase of lymph formation seems to be a factor to promote the oedema production, for any measure to increase the lymph formation only would never cause oedema. The increase in the flow of lymph cannot be referred to an alteration of vascular permeability due to the direct action upon the walls, for this could not be observed in the perfusion experiment and seems to be attributed rather to some alteration in the property of the blood. The increased imbibition of the tissues also might be of the same origin.

V. INFLUENCE ON THE BLOOD.

In the following experiments rabbits of about 2 kilos weight to which the last food had been given about twenty hours before the experiments were used in order to keep the blood in constant concentration during the performance. They were anaesthetized with urethane and tracheotomized. For the determination of the blood the minimum quantity of it was taken from a carotid artery, an external jugular vein or a posterior auricular vein only twice, once before the administration of paraphenylenediamine and again after the appearance of distinct oedema, in order to avoid as much as possible dilution of the blood due to bleeding.

1. Hydrogen ion concentration.

Tainter and Hanzlik, after measuring the concentration of hydrogen ions in the blood and the tissue fluid at the time of the formation of oedema, reported that no difference existed between the two, but they did not compare the concentration before and after the giving of paraphenylenediamine. Therefore I, myself, undertook this comparison in the blood.

3 c.c. of blood was diluted according to Cullen⁴ and its hydrogen ion concentration was estimated by Michaelis's colorimetric method⁵ using permanent indicator columnus. In the control experiment hydrogen ion concentration of the first sample was found not practically to differ from that of the second taken two hours after the first had been done.

Dealing with paraphenylenediamine it was observed that the value of pH of the blood taken at the height of the oedema was always lower than that of the blood taken before the administration of the poison, the difference being from 0.15 to 0.25, as is shown in Table II.

TABLE II.
Effect of paraphenylenediamine on pH of blood.

No. of experiment	Body weight (kilos)	Oedema	Before injection of p-phenylenediamine	After formation of oedema	Difference (%)
1	1.8	++	7.43	7.23	-0.2
2	1.9	+++	7.43	7.23	-0.2
3	2.0	+++	7.33	7.13	-0.2
4	2.2	+++	7.43	7.23	-0.2
5	2.2	+++	7.48	7.33	-0.15
6	1.8	+++	7.33	7.13	-0.2
7	2.0	+++	7.48	7.23	-0.25

2. Viscosity.

Hess' viscosimeter was used in estimating the viscosity of the blood. The viscosity of the blood taken at the height of oedema due to paraphenylenediamine markedly increased in every case, in comparison with that of the normal blood. The result obtained is presented in Table III.

TABLE III.
Effect of paraphenylenediamine on viscosity of the blood.

No. of experiment	Body weight (kilos)	Oedema	Before injection of p-phenylenediamine	After formation of oedema	Difference (%)
1	1.9	+++	3.6	5.2	44
2	1.8	+++	3.2	5.0	56
3	2.1	+++	3.8	5.2	37
4	1.8	+++	4.2	6.5	55
5	1.7	+++	3.6	6.2	72
6	1.8	++	4.0	5.4	35
7	1.9	++	4.0	5.3	33
8	2.0	+++	4.0	5.4	35

3. Suspension stability.

For the estimation Westergren's method⁶⁾ was adopted. In control experiments no essential difference in stability was observed between the blood samples taken at intervals of two hours from the same animal. In comparing the suspension stability before injection of paraphenylenediamine and after formation of distinct oedema it was found in every case that the latter was far greater than the former. This will be seen in Table IV.

TABLE IV.

Effect of paraphenylenediamine on suspension stability of blood.

No. of experiment	Body weight (kilos) & sex	Oedema		Sedimentation (mm.) in (hours)							
				1	2	3	4	5	24	48	
1	1.9 ♂	+++	Before After			1	1	2	6	15	
								3	5		
2	2.0 ♀	+++	Before After	1 0.7	2 1.3	3 2	4 2.5	5 3	16 7	36 10	
3	2.1 ♀	+++	Before After	1 0.7	2 1.5	3.5 2	5 2.5	5.5 3	8.5 5	20 8	
4	1.9 ♂	++	Before After			1 0.5	1.5	2 1	7.5 6	13 10	
5	2.5 ♀	+++	Before After	1	2	2.5 1		5 2	14 4	31 7	

Rabbits anesthetized with urethane and tracheotomized. 0.2 grm. paraphenylenediamine per kilo is injected subcutaneously.

Before.....Before injection of paraphenylenediamine.

AfterAfter formation of oedema from paraphenylenediamine.

4. Volume percentage of corpuscles in the blood.

About 0.1 c.c. of blood drawn was added to a small quantity of hirudin and absorbed in a haematocrit placed in a centrifugal machine in accordance with Hirota's direction,⁷⁾ and observed after a certain time. In control experiments the result of the second test conducted about two hours after the first showed in five different cases decreases in the volume percentage of corpuscles ranging from 2 to 5 per cent.

When paraphenylenediamine oedema developed markedly the volume percentage of corpuscles increased from 29 to 50 with an average of 39 per cent more than the value before injecting the poison, as is shown in Table V. This result coincides on the whole with that obtained by Tainter and Hanzlik.

TABLE V.

Total volume of red corpuscles in 100 parts of blood before and during oedema from paraphenylenediamine.

No. of experiment	Body weight (kilos)	Oedema	Total volume (%)		Difference (%)
			Before	During	
1	2.0	+++	40	57	+43
2	1.8	+++	42	54	+29
3	1.9	++	42	56	+33
4	1.6	+++	45	61	+36
5	1.8	+++	40	60	+50
6	2.1	+++	46	63	+37
7	1.6	+++	41	58	+41
8	1.5	+++	40	56	+40

5. Number of red corpuscles.

Thoma-Zeiss' haematocytometer was employed. In most cases of control experiments the number of red blood corpuscle contained in blood drawn at the second time was fewer than that drawn at the first, the difference being from 4 to 12 per cent.

At the stage where the oedema became very remarkable the concentration of red corpuscles increased without exception to a minimum of 14, a maximum of 46 and an average of 31 per cent, as indicated in Table VI.

TABLE VI.

Number of red corpuscles before and during oedema from paraphenylenediamine.

No. of experiment	Body weight (kilos)	Oedema	Number of red corpuscles (thousand)		Difference (%)
			Before	During	
1	1.6	+++	7060	8680	+23
2	1.8	+++	7120	9160	+29
3	1.6	++	7130	8220	+15
4	1.9	+++	5640	7920	+40
5	1.7	+++	7010	9970	+42
6	1.7	+++	5330	7810	+46
7	1.8	+++	5680	7990	+41
8	1.9	++	7280	8320	+14

6. Number of white corpuscles.

The method of experiment was the same as that used in the above. In four cases out of ten control tests the number of white corpuscles in the second sample was greater than that in the first and in the other cases smaller, the difference being only from 3 to 5 per cent.

At the height of the oedema, only the seventh case revealed an increase of 5 per cent in the number of white corpuscles, while all the others showed decreases of 10 to 45, averaging 21 per cent, as is to be seen in Table VII.

TABLE VII.
*Number of white corpuscles before and during oedema
from paraphenylenediamine.*

No. of experiment	Body weight (kilos)	Oedema	Number of white corpuscles (thousand)		Difference (%)
			Before	During	
1	1.8	+++	12.0	9.7	-19
2	1.7	+++	12.3	10.1	-18
3	1.8	+++	8.9	5.0	-44
4	1.9	+++	11.8	10.7	-9
5	2.0	+++	10.8	9.2	-15
6	1.8	+++	13.8	10.3	-25
7	1.8	++	9.9	10.4	+5
8	1.8	+++	10.4	6.6	-37

7. Sodium chloride content.

The estimation of sodium chloride contained in the blood was performed by the method of Ruszyna^k.⁵⁾ The quantity of blood required for each experiment was 0.13 c.c. Out of five control experiments there were two in which no difference in the content of sodium chloride between the first and the second samples, two in which the content in the second sample was greater than that in the first, and one in which the former was smaller than the latter. But the difference was very slight, being only 2 or 3 per cent.

TABLE VIII.
*Sodium chloride content of blood before and during oedema
from paraphenylenediamine.*

No. of experiment	Body weight (kilos)	Oedema	NaCl (gram)		Difference (%)
			Before	During	
1	1.9	+++	0.4815	0.4725	-2
2	1.9	+++	0.4815	0.4815	0
3	1.7	++	0.4365	0.4230	-3
4	1.8	+++	0.4050	0.4050	0
5	2.0	+++	0.4140	0.4095	-1
6	2.1	+++	0.4815	0.5040	+4
7	1.8	++	0.4815	0.4590	-5
8	1.8	+++	0.4455	0.4545	+2

Working with paraphenylenediamine no constant difference in the content of sodium chloride in the blood could be observed between the first and the second samples, showing increases in some cases and decreases in others. But the difference never exceeded 5 per cent, indicating that no remarkable change occurred in the sodium chloride content during the development of oedema. The result is presented in Table VIII.

8. Haemoglobin content.

Osgood and Huskin's standard solution⁹⁾ and Duboscq's colorimeter were used. The quantity of blood required was 0.05 c.c. in each experiment. Five control experiments showed in every case that the haemoglobin content in the second blood sample was smaller than that in the first, the difference being from 3 to 11, or on an average 7 per cent.

The results on injecting paraphenylenediamine were, as shown in Table IX, the reverse of those of the control tests, the second sample showing an increase of 24 to 44, averaging 33 per cent in the haemoglobin content as compared with the first. These results agree with those observed by Tainter and Hanzlik.

TABLE IX.

*Haemoglobin content of blood before and during oedema
from paraphenylenediamine.*

No. of experiment	Body weight (kilos)	Oedema	Haemoglobin content (%)		Difference (%)
			Before	During	
1	1.8	+++	116.9	162.0	+38
2	1.8	+++	114.5	151.4	+32
3	1.7	+++	102.5	139.9	+36
4	1.9	+++	124.4	160.0	+28
5	2.0	++	96.8	119.6	+24
6	1.8	+++	108.3	155.8	+44
7	1.7	+++	112.4	148.6	+32

9. Serum protein content.

Pulfrich's refractometer was used in estimating the serum protein. The quantity of blood taken in each case was about 0.2 c.c. In five control tests the content of serum protein in the second blood sample showed invariably decreases of from 3 to 8 per cent averaging 6 per cent as compared with that in the first.

In animals treated with paraphenylenediamine, the content of serum protein in the blood taken at the height of the oedema decreased to 2 to 12 per cent, the average however amounting only to 6.9 per cent, as indicated

in Table X. These results coincide with those obtained by Tainter and Hanzlik who stated that the percentage of protein before and after paraphenylenediamine, and during oedema remained practically the same.

TABLE X.
*Total protein content of blood serum before and during oedema
from paraphenylenediamine.*

No. of experiment	Body weight (kilos)	Oedema	Total protein content (%)		Difference (%)
			Before	During	
1	2.0	+++	6.8368	6.5520	- 4
2	1.8	+++	6.8320	6.5520	- 4
3	1.8	+++	7.4160	6.5520	-12
4	1.9	+++	6.2712	5.5750	-11
5	1.7	++	6.0110	5.7930	- 4
6	2.0	+++	6.1280	5.6840	- 7
7	2.1	+++	7.2000	7.0488	- 2
8	1.8	+++	5.9020	5.6840	- 4
9	1.7	+++	6.3360	5.5968	-12
10	1.8	+++	6.1200	5.5096	-10

10. Plasma protein content.

In this experiment rabbits which were not anaesthetized were used and 2 c.c. of blood was twice taken from the external jugular vein at an interval of two hours. The ratio of proteins in the plasma was estimated by Ruszynek's method.¹⁰⁾ The results of five different control tests showed that albumin content was on the whole the greatest amounting to 70 to 80 per cent, the globulin content the next amounting to 16 to 28 per cent and the fibrinogen content the smallest amounting only to 1 per cent. Between the first and second drawings of blood almost no difference was observed in the albumin content, and a slight decrease in the globulin and just a tendency to decrease in the fibrinogen were observed in the second drawing. The total protein content in the plasma which was separated from a mixture composed of citrate-plasma, i. e., sodium citrate and blood in the ratio of 1 to 4, was measured refractometrically and some decreases in the second sample were observed in every case.

The results of ten experiments carried out before and after the formation of oedema due to paraphenylenediamine are presented in Table XI. During the developement of oedema, the albumin content slightly increased only in the third and seventh tests, while it decreased 9 to 21 per cent in the remaining cases. The globulin content showed an inverse change decreasing slightly in the third and the seventh tests, while making a marked

TABLE XI.
Simultaneous determination of protein fractions and total protein content of blood plasma before and during oedema from paraphenylenediamine.

No. of experiment	Body weight (kilos)	Oedema	Fibrinogen (%)			Globulin (%)			Albumin (%)			Total Protein (%)		
			Before	During	Difference (%)	Before	During	Difference (%)	Before	During	Difference (%)	Before	During	Difference (%)
1	2.0	++	0.7	5.0	+674	22.3	28.0	+26	77.0	67.0	-13	4.5	4.1	-8
2	2.5	++	0.5	1.7	+240	12.3	20.0	+62	87.2	78.3	-10	4.9	4.0	-18
3	2.0	++	0.6	2.6	+333	17.8	14.0	-21	81.6	83.4	+2	5.0	4.2	-18
4	2.2	++	0.7	2.1	+200	18.5	29.1	+57	80.8	68.8	-14	5.2	4.6	-11
5	2.8	++	0.9	2.2	+144	17.6	25.7	+46	81.5	72.1	-11	5.0	4.6	-8
6	2.4	++	0.7	3.6	+414	17.8	22.7	+27	81.5	73.7	-9	5.2	4.9	-5
7	2.0	++	0.5	0.7	+40	16.7	15.9	-5	82.8	83.4	+0.7	5.0	4.5	-10
8	2.5	++	0.7	2.9	+314	20.1	35.1	+74	79.2	62.0	-20	5.4	5.0	-7
9	2.3	++	1.0	3.8	+280	19.0	26.2	+37	80.0	70.0	-12	5.2	4.8	-7
10	2.3	++	1.2	6.3	+425	29.8	37.7	+26	69.0	56.0	-18	5.7	4.9	-14

0.2 grm. Paraphenylenediamine per kilo is injected subcutaneously.

increase, reaching a maximum of 74 per cent, in the others. As for the fibrinogen content a great increase occurred in every case and in some cases it became seven times greater. The total protein content in the citrate-plasma slightly decreased in every test.

It is noticeable that during the development of oedema the ratios of plasma proteins undergo changes, the fibrinogen content strikingly increasing in every case, while the albumin content decreasing and the globulin content increasing in the cases in which distinct oedema occurs and reversing the order in the cases in which the oedema is not very marked.

11. Blood sugar content.

The method employed for the estimation of the sugar content in blood was Hagedorn's.¹¹⁾ In six control experi-

ments the sugar content of the second samples were always greater than that of the first, the difference being however only from 2 to 4 per cent.

The sugar content in the blood drawn at the height of the oedema increased in every case, the increase amounting to a minimum of 21, a maximum of 61 and an average of 46 per cent, as is shown in Table XII.

TABLE XII.
*Sugar content of blood before and during oedema
from paraphenylenediamine.*

No. of experiment	Body weight (kilos)	Room temper- ature °C	Oedema	Blood sugar (%)		Difference (%)
				Before	During	
1	2.0	21	+++	0.12	0.19	+58
2	2.1	21	++	0.09	0.13	+44
3	2.0	22	+++	0.13	0.21	+62
4	1.8	21	+++	0.12	0.19	+58
5	1.7	21	++	0.14	0.17	+21
6	2.0	20	+++	0.12	0.17	+42
7	1.7	22	++	0.13	0.18	+38

12. Non-protein nitrogen content.

Kowarski's simplified method¹²⁾ was adopted in this experiment. Five control tests showed a decrease in the second blood sample, the average being a decrease of 5 per cent.

After the formation of oedema the non-protein nitrogen content of the blood increased from 14 to 35, averaging 23 per cent. The results are presented in Table XIII.

TABLE XIII.
*Non-protein nitrogen content of blood before and during
oedema from paraphenylenediamine.*

No. of experiment	Body weight (kilos)	Oedema	Non-protein nitrogen (mgrm.)		Difference (%)
			Before	During	
1	2.0	+++	45.7	57.7	+27
2	2.0	++	36.0	42.0	+17
3	2.1	+++	37.3	50.3	+35
4	1.6	+++	34.5	39.7	+15
5	1.8	++	41.7	47.4	+14
6	1.9	+++	35.4	46.2	+30
7	1.6	+++	40.0	49.7	+24

So far in testing the changes in the blood occurring at the time of the formation of intense oedema, an increase in the hydrogen ion concentration, the viscosity, the suspension stability, the number of red blood corpuscles and a decrease in the number of white blood corpuscles were confirmed. No marked change occurred in the sodium chloride content or the total protein content of the serum, but an increase in the haemoglobin content. And although there was no marked change in the total protein content of the plasma, its albumin content was considerably decreased, while on the contrary the globulin and fibrinogen contents were greatly increased. Besides these an increase was also brought about in the blood sugar content and the non-protein nitrogen content.

The increase in the viscosity, suspension stability and so on seems to be caused by accelerating the lymph formation. That the sodium chloride content and the total protein content of the serum suffer almost no change indicates that the plasma escapes as it is out of the blood vessels, as assumed by Tainter and Hanzlik. Since, as I have already mentioned, the acceleration of lymph formation cannot be referred to the action of the poison in increasing the permeability of the blood vessels, and accordingly its cause must be sought in some alteration occurring in the blood composition. Though it was impossible to find any such alteration in the blood of animals treated with paraphenylenediamine, it is however possible it occurs, considering that during the oedema the blood undergoes several changes which are not the result of the acceleration of lymph formation. The increase in the imbibition of the tissues might probably have a similar cause.

VI. EFFECT ON THE URINE SECRETION.

Since in the great majority of the above experiments animals treated with paraphenylenediamine showed no urination during the course of observation, experiments were performed in order to ascertain whether this is due to the action of the poison inhibiting the urine formation. As expected, it was found that in rabbits anaesthetized with urethane, tracheotomized and injected with the usual dose of the poison the quantity of urine flowing out of a cannula connected with a collector inserted into the bladder began to decrease some thirty or forty minutes before the oedema was noticed on the tongue, and continued gradually to do so until the flow almost completely or completely stopped when the oedema became very marked. Considering the time relation between the urine diminution and the oedema formation there is no doubt that the former is a natural consequence of the

concentration of the blood. Yet it is not clear whether this is to be regarded as the only cause of the urine diminution. That the renal function is not affected by the poison is stated by Tainter and Hanzlik who failed to find any definite change in the secretion of phenolsulphone-phthalein from the kidneys or any histological change in the kidney tissue. However to be certain whether renal injury also plays some rôle in it. I made an experiment on the kidney excised from a rabbit in order to observe the action of the poison upon its secretion.

An excised kidney was perfused with Ringer's solution, containing acacia, warmed and oxygenated. When it reached a stage in which the quantity of fluid flowing out of the vein and of the ureter very slowly and almost constantly decreased, the perfused fluid was replaced by another containing paraphenylenediamine in a concentration of 0.005 per cent which is not sufficient to produce any constrictory effect on the vessels. This was immediately followed by a decrease in the flow from the ureter, as is shown in Table XIV.

TABLE XIV.
Perfusion of the kidney.

Outflow from vein per minute (c.c.)	Outflow from ureter per minute (drops)	Remarks
5.5	3	Exchange for the solution containing 0.005% paraphenylenediamine
5.4	4	
5.4	3	
5.4	4	
5.3	4	
5.4	3	
5.3	2	
5.2	2	
5.3	1	
5.3	0	
5.2	0	
5.2	1	
5.2	0	
5.1	0	
5.1	1	

This result shows that paraphenylenediamine inhibits the fluid passing through the kidney by its direct action on the organ. Although this may not be an important factor in the diminution of urine secretion, it probably plays some part.

SUMMARY.

1. Experiments performed on rabbits with paraphenylenediamine show that the poison has an action to cause oedema over many tissues of the body, this appearing however especially prominently on the head and neck.

2. The oedema is not prevented by atropine at all, indicating that no relation exists between the oedema formation and the parasympathetic nerves. The injection of a concentrated calcium chloride solution prevents the oedema formation by its salt action, just as that of sodium chloride solution does, but not by its special action.

3. The poison causes a rise in the blood pressure by its action on the heart, which may facilitate the oedema formation to some extent. In perfusion experiments on the excised ear, the tongue and kidney failed to prove any increase in the vascular permeability due to the poison.

4. The poison increases the lymph formation, but at the stage where the oedema is developing the lymph flow temporarily diminishes owing to an increase in the imbibition of the tissues, which would be regarded as the cause of oedema.

5. During the oedema the blood undergoes several changes, partly due to the increase of lymph formation, but partly independent of this. This fact seems to lead us to conceive that the increase in the formation of lymph and in the imbibition of tissues is due to some alterations which may occur in the blood.

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