

THE EFFECT OF THE INTRAVENOUS INJECTION OF
SUBSTANCES AFFECTING TUMOR GROWTH ON
THE CYCLIC CHANGES IN THE OVARIES AND
ON PLACENTOMATA.*

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In preceding communications¹ we described the effect of the intravenous injection of various substances on tumor growth and on the liver of guinea pigs. In the present report we shall compare with the data obtained in these studies, the effect of the intravenous injection of some of these substances on experimentally produced placentomata, which represent rapidly growing tumor-like formations, and on the cyclic changes of the ovaries.

THE EFFECT OF THE INTRAVENOUS INJECTION OF HIRUDIN ON
THE OVARIES.

In four experiments hirudin was injected intravenously² into female guinea pigs on the ninth or tenth day of the sexual cycle, and the animals died within a short time of the injection (four and one half to seven and one half hours after the injection; in one case the exact time of death was unknown, but the animal died within the first twenty-four hours). Six to seven days after ovulation, incisions had been made, under ether anesthesia, in the uterus. In these cases no marked changes were found in the ovaries, although, as we shall see later, a decided effect had been produced on the placentomata. There was perhaps a slightly increased degeneration of the large follicles, but it was not significant. There was in some cases an increase in the number of polynuclear leucocytes in the corpora

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¹ Fleisher, M. S., and Loeb, L., *Jour. Exper. Med.*, 1914, xx, 169. Loeb L., and Fleisher, M. S., *Jour. Am. Med. Assn.*, 1913, lx, 1857.

² 4 to 8 c.c. of a 0.25 per cent. solution of hirudin were injected each time.

lutea, mainly in the capillaries, but in some cases the leucocytes had entered the lutein cells.

In two cases the ovaries were examined one day after the injection of hirudin. In one of the cases the large and medium sized follicles and some of the small follicles showed degeneration of the granulosa. A mature follicle was, however, well preserved. In the corpus luteum there was some vacuolization in the periphery. In this case incisions had been made into the uterus six and one half days after ovulation, and three days later the hirudin was injected. In the other case hirudin was injected intravenously in a pregnant guinea pig. In this case we found also in all the large and medium follicles degeneration of the granulosa, while the mature follicles, as well as the small follicles, were well preserved. The capillaries of the corpora lutea were crowded with polynuclear leucocytes to such an extent that some of the capillaries appeared occluded; some lutein cells were necrotic. In a third case, in which without previous incisions into the uterus, hirudin had been injected six and one half days after copulation, the ovaries were normal.

We may conclude that within the first twenty-four hours after injection of hirudin the degeneration of the granulosa sets in at first in all the large and medium sized follicles and subsequently also in some small follicles. This degeneration of the granulosa is followed by ingrowth of connective tissue and complete atresia of the follicles. The mature follicles are considerably more resistant than the other large follicles,³ and in a similar manner as they survive the injurious effect of those conditions which lead to ovulation, while the other follicles perish at that time, they may also survive the effect of hirudin, to which the other follicles succumb. We may find a large number of polynuclear leucocytes in the corpora lutea and also some degeneration of the lutein cells. At the same time we see that hirudin does not in all cases have this effect on the ovaries.

If about seven days after copulation incisions are made, under ether anesthesia, into the uterus, three days later hirudin is injected, and two or three days after the injection the ovaries are examined, we find only good follicles of small or small to medium size, while

³ Loeb, L., *Centralbl. f. Physiol.*, 1911-12, xxv, 336; *Jour. Morphol.*, 1911, xxii, 37.

the large majority of follicles are found to be in various stages of connective tissue atresia. There may still be found some large follicles undergoing degeneration of the granulosa. In the corpora lutea we may or may not find collections of polynuclear leucocytes and some vacuolization and karyorrhexis in the lutein cells. In another case, however, in which nine days after copulation the hirudin had been injected without previous incision into the uterus, no changes were found in the ovaries.

In a further series of experiments two or three injections of hirudin were given in succession. In the majority of cases the incisions into the uterus were made six to seven days after copulation; three and five days following the operation hirudin was injected. The ovaries were examined from a few hours to three days after the last injection. In all these cases we find approximately the same picture in the ovaries; namely, good follicles of small or small to medium size and follicles in various stages of connective tissue atresia. There are especially many follicles in the last stage of connective tissue atresia; in one case some large follicles with degenerating granulosa were still present. In several other cases mature follicles were seen in which degeneration of the granulosa had set in. As we mentioned previously, mature follicles are very resistant, but they also degenerated under the influence of several injections of hirudin. In the corpora lutea we often found dilated vessels; polynuclear leucocytes might be present or absent. Some vacuolization of the lutein cells could sometimes be observed in the corpora lutea of these animals; it may, however, begin to set in normally at this period of the sexual cycle. The same changes were found in cases in which three instead of two injections of hirudin had been given.

In two cases of pregnancy where, without previous incisions into the uterus, two injections of hirudin were given, the changes were not as definite in the ovaries as in the previous experiments. In one of these two pregnant animals abortion followed the injection of hirudin.

From these experiments we may conclude that if six to seven days after copulation incisions are made into the uterus under ether anesthesia, and on the following days one or several injections of

hirudin are given, changes set in in the ovaries which correspond to those found at the time of ovulation; namely, a degeneration of all with the exception of the small follicles. These degenerative changes are followed by the same developmental changes which one of us has described previously in the ovaries.⁴ In the cases that were observed for a certain period of time following the injection, the ovaries gradually resumed their normal function and ovulation occurred again. The corpora lutea are also sometimes, but not always, affected as a result of these injections. We found thus a second method of altering experimentally the periodicity of the sexual cycle; the first one consisted in the early extirpation of the corpora lutea, which one of us has described previously.⁵ In several cases in which the injection of hirudin was given without previous incisions into the uterus, the ovaries were not at all, or were only slightly affected.

THE EFFECT OF THE INTRAVENOUS INJECTION OF HIRUDIN
ON THE PLACENTOMATA.

In the majority of the guinea pigs referred to in the previous paragraph incisions had been made into the uterus of the anesthetized animals approximately five and one half to seven days after copulation. This operation was followed within the next week by one or several intravenous injections of hirudin. As a result of the incisions into the uterine wall placentomata began to develop within a few days after the operation. We were, therefore, in these experiments, in a position to determine the effect of hirudin on the placentomata.

In four animals in which the hirudin was injected about three days after the operation and in which the placentomata were examined, in three cases four and one half to seven hours, and in one case within the next twenty-four hours following the injection, as a result of the injection of hirudin by far the greater part of the developing placentomata had been destroyed by hemorrhage; however, small placentomatous areas near the mucosa had been pre-

⁴ Loeb, L., *Jour. Morphol., loc. cit.; Virchows Arch. f. path. Anat.*, 1911, ccvi. 278.

⁵ Loeb, L., *Deutsch. med. Wchnschr.*, 1911, xxxvii, 17.

served; in the preserved areas there may have been some hyperemia; the number of mitoses in the cells of the placentomata was either very small or mitoses were entirely missing. The adjoining mucosa of the uterus was occasionally found to be edematous.

In two animals in which the placentomata were examined one day after the injection of hirudin, necrosis had set in in the greater part of the placentomatous tissue as a result of interference with the nourishment following the hemorrhages. In the majority of animals the placentomata were examined at a later period, about six to seven days after the operation, after one or several injections of hirudin had been given and several days after the last injection of hirudin. In these cases we noticed on macroscopical examination that the placentomata were much reduced in size; in some cases it was difficult to decide whether placentomatous tissue developed at all. Microscopically we find most of the placentomatous tissue destroyed by hemorrhage and necrotic. There were usually present some small islands of living placentomatous tissue which were situated near the mucosa, at places where the circulation had been less interfered with. But even in these areas, as well as in the adjoining mucosa, there were present indications of passive congestion. In the living placentomatous tissue mitoses were either missing or very few in number. The unfavorable circulatory conditions prevented probably a very active cell proliferation under those conditions. Hemorrhages could occasionally also be seen in the adjoining mucosa. In some cases collections of polynuclear leucocytes were present in the necrotic areas. They were in part attracted by the substances given off by the necrotic tissue. It is, however, probable that at least occasionally bacterial infection played a part. We found in some cases in which we examined the animals after injection of hirudin that certain bacteria developed at the place of injection; and even in the heart blood and in necrotic areas of the liver produced through these injections, bacteria could be discovered after intravenous administration of hirudin, while after injection of colloidal copper bacteria could not be found under those conditions. It is therefore possible that bacteria were retained in the necrotic area of the placentomata, and formed an additional attraction for the leucocytes. We observed one case in which hirudin failed to exert its usual effect on the placentomata.

In four cases the hirudin was once or twice injected at a certain stage after copulation without preceding incisions into the uterus. In two of these cases, early cases of pregnancy about ten to thirteen days after copulation, no abnormality could be found in the pregnant uteri; in two other cases in which the pregnancies were somewhat further advanced, hemorrhage took place in the neighborhood of the placenta, as a result of the injections of hirudin, with the subsequent death of the embryo in one case. We may assume that in both cases abortion would have taken place as a result of the injection, if the guinea pigs had been permitted to live for a longer period after injection of hirudin.

We may then conclude that intravenous injections of hirudin destroy in the majority of cases the greater part of the placentomata through hemorrhage, and they prevent the remaining placentomatous areas from active proliferation, probably also as a result of interference with the circulation. They may cause abortion in pregnant animals. While in the case of the placentomata hemorrhages are mainly responsible for the destructive action of hirudin, the action of hirudin on the ovarian cycle must have a different cause. Hemorrhages are missing in the ovaries. It appears probable that interference with the nourishment of the follicles as a result of the action of hirudin on the blood, or on the exchange of substances between blood vessels and tissue cells, is responsible for this effect.

THE RELATION BETWEEN INCOAGULABILITY OF THE BLOOD AND A TENDENCY TO HEMORRHAGE.

Our observations on the effect of hirudin on the placentomata suggest some general considerations on the possible relation between a tendency to hemorrhage and a decrease in the coagulability of the blood. In cholemia, in hemophilia, and in purpura such a connection exists. Morawitz and Bierich⁶ found that if the blood of a dog is made free of fibrinogen and the blood coagulation is retarded, a hemorrhagic diathesis is not observed. These authors come therefore to the conclusion that there exists no direct connection between

⁶ Morawitz, P., and Bierich, R., *Arch. f. exper. Path. u. Pharmakol.*, 1907, lvi, 115.

the retardation of the coagulation of the blood and the tendency to hemorrhage.

In our observation we find another instance of a parallelism in the retardation of the coagulation of the blood and in the tendency to cause hemorrhages in the case of hirudin. Intravenous injection of hirudin almost invariably causes hemorrhages into the placentomata. It frequently causes hemorrhages in mouse tumors after injection into the vein of the tail of the mouse. We stated in a previous paper⁷ that hirudin frequently leads to necrosis in the liver of guinea pigs, and that in the neighborhood of this necrosis, hemorrhages may be found. We did not make a systematic study of other changes produced in the body through intravenous injections of hirudin, but we noticed in several cases erosions or ulcers and some hemorrhages in the wall of the stomach of guinea pigs, and in another case we found hemorrhage in the kidney tissue, blood casts and hyaline casts in the tubules, and some hyperemia of the glomeruli of the kidney. We may therefore conclude that injection of hirudin produces a tendency to hemorrhage in various parts of the body, and that the hemorrhages are prone to occur especially at such places where the blood vessels are not well formed, or are less resistant, as in tumors and in placentomata, or in the neighborhood of necrotic areas; and that in the stomach hemorrhages may be followed by digestion of the tissue through the gastric juice. It appears probable that there is a connection between the action of hirudin on the blood (effect on the coagulability or on the viscosity) and the tendency to hemorrhage which we noticed.

THE EFFECT OF VARIOUS OTHER SUBSTANCES ON THE OVARIES AND ON PLACENTOMATA.

A number of control experiments were carried out. We found changes in the ovaries after injection of hirudin especially in animals in which placentomata had been produced and in which hemorrhages into the placentomata had taken place. It was therefore conceivable that loss of blood in itself might produce some changes in the ovaries.

In each of three guinea pigs five cubic centimeters of blood were

⁷ Fleisher and Loeb, *loc. cit.*

withdrawn from the jugular vein twice, an interval of two days elapsing between the first and second bleeding. On examination three days after the last bleeding, the ovaries and uterus were found to be normal in two animals; in the third animal the ovaries were hypotypical.⁸ There were small and small to medium sized good follicles, many follicles in early and medium connective tissue atresia, there were many contracted follicles, and yellow scar-like bodies, the remains of former corpora lutea. The uterus showed low cuboidal epithelium in surface and glands; there were a few mitoses in the surface epithelium. The connective tissue of the mucosa was fibrillar and contained small nuclei. Some small mononuclear cells migrated from the connective tissue into the surface epithelium. It is not probable that the bleeding is responsible for the hypotypical changes in the ovaries of one of these animals and we may conclude that withdrawal of blood is without marked effect on ovaries and uterus.

In four anesthetized guinea pigs in which incisions into the uterus had been made about seven days after copulation, ten cubic centimeters of distilled water were injected intravenously two to nine days later. The examination was made one to four days following the injection. In three cases ovaries and placentomata were normal; in one case in which the animal was not in good condition one day after the injection, degeneration of the granulosa occurred perhaps somewhat earlier than usual, in some follicles of medium size. In a rabbit in which also placentomata had been produced experimentally, intravenous injection of distilled water had no influence on the placentomata. No effect was obtained through injection of distilled water in guinea pigs in which no incisions had previously been made into the uterus; one day after the injection the ovaries and uterus were found to be normal. We may conclude therefore that intravenous injection of distilled water has no distinct influence on the ovaries and uterus in the guinea pig.

In six guinea pigs the effect of repeated injections of colloidal solutions of copper on the ovaries and placentomata were tested. Six to seven days after copulation incisions into the uterus of the anes-

⁸ Loeb, L., *Centralbl. f. Physiol., loc. cit.*; *Virchows Arch. f. path. Anat., loc. cit.*

thetized animals were made, and in four cases four, in one case three, and in the last case two injections of colloidal copper were given. Ten cubic centimeters of the solution were injected each time. The first injection was given three or four days after the uterine operation; the following injections were made on the following days, and the organs were usually taken out for examination one day after the last injection. The ovaries were found to be normal in all cases examined; in one case, however, all the large follicles showed degeneration of the granulosa. It is doubtful whether this was due to the influence of the colloidal copper. The placentomata in these cases were much larger than in the animals which had received hirudin; their size was approximately normal. There was, however, in most cases some hemorrhage and necrosis in the placentomata and it is possible that the injections of colloidal copper increased the hemorrhage and necrosis in the placentomata, which to some extent may be found even in guinea pigs that had not received any injection. There was, however, usually a considerable amount of living placentomatous tissue present with numerous mitoses. In two rabbits which had been similarly treated good deciduomata were found, and in one case a normal pregnancy was present.

We may conclude that repeated intravenous injections of colloidal copper are without effect on the ovaries of guinea pigs and without marked effects on the placentomata of guinea pigs and rabbits. They may possibly increase somewhat the amount of hemorrhage and necrosis, often to some extent normally present in further developed placentomata.

In twelve guinea pigs either one or two intravenous injections of nucleoproteid prepared from the kidney or lymph glands of cattle were given after incisions into the uterus of the anesthetized animals had been made about six to seven days after copulation. The first injection was given two to five days (in one case nine) after the operation; the second injection one to two days after the first one; two cubic centimeters of a 15 per cent. solution were given each time. Ovaries and uterus were in most cases taken out one to three days after the last injection. In the majority of cases the ovaries were found to be normal. In one case, however, one day after the injec-

tion there was much degeneration of the granulosa, even in medium sized and in some small follicles; in two other cases large follicles without degeneration of the granulosa were missing. It is therefore possible that in a few cases a slight effect on the ovaries was obtained; but in the majority of cases no effect was present. The placentomata were not noticeably influenced through the injection of nucleoproteid; occasionally some hemorrhages were observed in the placentomatous tissue.

In four guinea pigs in which six to seven days after copulation incisions into the uterus of the anesthetized animals had been made, nine to ten days after the operation intravenous injections of ten cubic centimeters of a 1 $\frac{2}{3}$ per cent. solution of casein were given. The placentomata in three cases were taken out one day later; in one case the animal died ten minutes after the injection; in two cases there were extensive hemorrhages and necroses in the placentomata. In the two other cases the placentomata were in good condition. In two cases in which the ovaries were examined they were found to be normal. We have to consider the possibility that at this late stage of development of the placentomata (nine days after the operation) even mechanical factors (perhaps handling of the animals during intravenous injection) may be liable to cause some hemorrhage. In a rabbit injections of casein had no noticeable effect on the experimental placentomata or on pregnancy.

We may conclude that the various experimental interferences mentioned do not produce any marked or constant effect on the ovaries or on the placentomata.

SUMMARY.

If six to seven days after copulation incisions are made under ether anesthesia into the uterus, and on the day following this operation one or several injections of hirudin are given, changes set in in the ovaries which correspond to those found at the time of ovulation; namely, a degeneration of all the follicles with the exception of the small ones. These degenerative changes are followed by the same developmental changes as in the normal cycle. This represents a second method of altering experimentally the periodicity of the sexual cycle, the first consisting in the early extirpation of the corpora lutea described previously by one of us.

Under the same conditions intravenous injections of hirudin destroy in the large majority of cases the greater part of experimental placentomata through hemorrhages, and they prevent the remaining placentomatous areas from active proliferation, probably as a result of interference with the circulation. These injections may also cause abortion in pregnant animals.

Intravenous injections of hirudin produce a tendency to hemorrhage at various places in the body, and these hemorrhages are prone to occur, especially in rapidly growing tissues, where the blood vessels are less resistant, as in tumors and in placentomata, also in the neighborhood of necrotic areas. In the stomach the hemorrhages may be followed by digestion of the tissues through the gastric juice. It is probable that there is a connection between the action of hirudin on the blood (coagulability and viscosity) and the tendency to hemorrhage.

Withdrawal of blood, intravenous injections of distilled water, colloidal copper, nucleoproteid, or casein have no marked effect on the cyclic changes in the ovaries or on placentomata.