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# THE RESPONSE OF THE PERITONEUM TO THE INSUFFLATION OF GIANT RAGWEED POLLEN

It has been known for many years that pollens are capable of producing an allergic response in man, either as a seasonal rhinitis or asthma. There are many questions concerning allergies that are left to be answered in the future. The findings described in this paper are an attempt to approach the problem from a new angle. For this study insufflation of pollen was carried out intraperitoneally in rats and guinea pigs. Giant ragweed pollen was chosen because it is probably the greatest single cause of seasonal rhinitis.

The antigen antibody reaction is the basis for most of the theories of allergy. The antigens being the stimulus required to produce the antibody and also the factor responsible for the allergic response. Antibodies are proteins similar in all respects to serum globulin varying only in molecular arrangement. It is thought that antigens disturb the normal formation of globulin to form abnormal globulins or antibodies. If the antigen antibody reaction occurs in the cells the following substances are believed to be liberated; histamine, heparin, adenosine derivities, acetylcholine, potassium ions and many other substances as yet unknown.[2] If this reaction is on a systemic basis, anaphylaxis occurs. Anaphylaxis varies with the species of an animal.[3] In the guinea pig tetanic contractions of the smooth muscles of the bronchials inhibit the expiration of air so the guinea pig dies

with inflated lungs. With a rabbit the pulmonary circulation is obstructed because of tetanic contractions of the smooth muscles of pulmonary vessels. In a dog, shock is caused by the vaso dilation of all the capillaries of the liver so that sixty per cent of the blood is found in the liver. The rat is more resistant to fatal anaphylaxis unless either the adrenal or the hypophysis has been removed.[4] The pathological changes consist of venous congestion, edema, hemorrhage and eosphinilia. This is more extensive in the upper intestinal tract.[5]

When the reaction occurs in the skin of humans it is either urticaria or allergic dermatitis. In animals, if the reaction persists long enough, it produces the arthus reaction. This requires twenty-four hours for the maximal expression but begins to take effect immediately.[2] The effect seems to come from damaged vessels rather than local cells. Pathological findings are hyperemia and edema. A third factor seems to come into play namely the reagin. Reagin is the result of a distorted or incomplete antibody formation forming a unipolar antibody.[6] This reagin formation in the body is probably the reason that the skin reaction does not disappear in humans by desensitization since the antibody is reduced but not the reagin.[2]

Guinea pigs have been sensitized by using a 7% extract of defatted short ragweed pollen in Coca's alkaline fluid without phenol using 1/9th volume of 10% potassium alum with enough N/10 sodium hydroxide to neutralize. One tenth cc was injected twice

weekly for three weeks to produce sensitization.[7]

There are various methods of preparing extracts of pollens. The old method was by extracting the antigenic properties in glycerin. This produces a stable compound but is very painful on injection. Other methods are alkaline extracting fluid (Coca's), buffered saline extracting fluids (Evan's), and buffered sodium formaldehyde sulfoxylate extracting fluid (Strauss and Spain).[8]

The major portion of this experiment revolves around intraperitoneal insufflation of giant ragweed pollen. This was done using a large sixteen guage bone marrow biopsy needle. This was injerted through anterior abdominal wall of the experimental animal after shaving and painting the area with alcohol. The pollen was measured into a 1 cc tuberculin syringe. A rubber syringe bulb was fitted over the end of the tuberculin syringe and the pollen blown into the peritoneal cavity. For this work 0.1 cc of the pollen was used which weighed approximately twenty-five mgms. Thus the only trauma inflicted was at the sight of the needle puncture, since the air was allowed to escape through the needle before the stylet was reinserted and the needle withdrawn.

The purpose of this experiment was two fold:

1. To determine what happened to the pollen.
2. What is the difference in the reaction between the sensitive and the non-sensitive animal.

Therefor, we first had to prove that animals could be sensitized

to giant ragweed pollen and its extract. The extract used was a 5% giant ragweed pollen in Coca's solution.

The first work was done with rats. Originally the ragweed pollen was blown into the peritoneum and skin tests were attempted approximately every other day. The skin tests were intradermal injections of 0.1 cc of the extract. A control rat #7 without the insufflated pollen was tested in the same manner. The thirteenth day following the installation of pollen, an intracardial injection of 1/2 cc of the 5% extract was given which produced a severe shock terminating fatally in one hour and forty minutes, rat #8. The control rat #7 was injected in the same manner; fatal shock resulting in five minutes. A third rat #2 was given 0.25 cc intracardially on the fourteenth day following ragweed pollen insufflation with only a mild shock. The following day 0.5 cc was given intracardially resulting in a fatal shock. This rat had four skin test doses during this period. The fourth rat #3 was given an intraperitoneal injection of 0.5 cc of the extract on the fifteenth day with no effect. Two days later 0.5 cc intracardial injection produced no effect. During the fifteen day interval this rat had four skin tests. This rat was killed thirty days following the peritoneal insufflation of pollen. The fifth rat #1 was injected with 0.2 cc of the extract intracardially producing only a paling of the ears and feet and a transitory dyspnea which disappeared within ten minutes. A repeated injection three days later with 0.5 cc of the extract produced the same type of reaction.

Three days following this injection the animal was reinjected in the same manner. This terminated fatally in five minutes during convulsions. Autopsy however showed pericardial cavity to be filled with blood. The above tests seemed to indicate that the rats were sensitive to the extract but did not prove sensitivity to the insufflated pollen. Therefor other rats were insufflated with the pollen and no skin tests were given. It was found in these animals that some effect could be obtained from the fifteenth to the twenty-second day, table I. Four types of shock were

TABLE #1

| Number | Method of Sensitization | Time Interval | Result |
|---|---|---|---|
| # 5 | Insufflation* | 15 days | Fatal |
| # 19 | Insufflation | 17 days | Fatal |
| #10 | Insufflation | 17 days | Mild |
| # 1 | Insufflation | 20 days | Moderate |
| # 9 | Insufflation | 22 days | Marked |
| #14 | Insufflation | 28 days | No effect |
| #13 | Control | --- | No effect |
| # 4 | Control | --- | No effect |
| #18 | Control | --- | No effect |

*Insufflation indicates approximately 25 mgm. intraperitoneally.

produced:

1. Mild shock which consisted of a plaing of the ears and feet, a transitory dyspnea which persisted more than ten minutes.
2. Moderate shock consisting of apaling of the ears and feet, prolonged dyspnea and some loss of muscle control. This required one to one and one-half hours for recovery.

3. Marked shock, paling of the ears and feet, dyspnea, complete loss of muscular control, urination and deficiation. The recovery period usually exceeded two and one-half hours.

4. Fatal shock. This usually occurred within ten minutes with the only exception being rat #8.

This period of sensivity does not coincide with the finding of others who have found rats to be sensitive.[4] They list the period of sensivity as the tenth to the fifteenth day. However, this may be due to the fact that they use serum as an antigen whereas ragweed pollen may require several days for the antigenic properities to be absorbed in sufficient quanities to produce antibodies.

After proving to our satisfaction that white rats could be sensitized to ragweed pollen, the experiment was begun to note the difference in reaction to pollen insufflated into sensitive and non-sensitive animals. After doing a series of rats,* another series was done with guinea pigs. Since the rat might not be the animal of choice for this experiment, as it is well known that rats do not react as well to antigens as guinea pigs.[9] Therefor, a series of guinea pigs were sensitized in the same manner. The method used to sensitize the animals was three daily intradermal injections of 0.2 cc giant ragweed extract.

At this time it was decided that perhaps more positive proof of sensivity, than our production of shock, was required. The



*Table #2

TABLE #2

| Number of Animals | Time Interval Following Sensitization | Time intervarl between Insufflation and death |
|---|---|---|
| 2 | 13 days | one-half hour |
| 2 | control | one-half hour |
| 2 | ten days | one hour |
| 2 | control | one hour |
| 2 | ten days | two hours |
| 2 | control | two hours |
| 2 | 14 days | three hours |
| 2 | control | three hours |
| 2 | 15 days | four hours |
| 2 | control | four hours |
| 2 | 16 days | six hours |
| 2 | control | six hours |
| 2 | 16 days | seven hours |
| 2 | control | seven hours |
| 2 | 22 days | eight hours |
| 2 | control | eight hours |
| 2 | 22 days | twelve hours |
| 2 | control | twelve hours |
| 2 | 22 days | twenty hours |
| 2 | control | twenty-hours |
| 2 | 22 days | twenty-four hours |
| 2 | control | twenty-four hours |
| 2 | 23 days | twenty-eight hours |
| 2 | control | twenty-eight hours |
| 2 | 22 days | thirty-two hours |
| 2 | control | thirty-two hours |
| 2 | 22 days | thirty-six hours |
| 2 | control | thirty-six hours |

TABLE #3

| Number of Guinea Pigs | Time Interval Following Sensitization | Time Interval between Insufflation and death |
|---|---|---|
| 2 | 20 days | twenty-four hours |
| 2 | control | twenty-four hours |
| 2 | 21 days | thirty hours |
| 2 | control | thirty hours |

feeling being that other factors might be producing the reaction noted. These factors might be:

1. Intracardial injections as the cause of death either by damage to the blood supply or nerve conduction system of the heart.
2. The possibility of overtaxing the cardio-vascular system with the injection of the extract.
3. The possibility of hemolysis due to the fact that the extract is not isotonic.

However, no reactions were obtained in any of the controls.

Six virgin guinea pigs were injected with the three daily sensitizing injections. These were sacrificed on the fourteenth, fifteenth and sixteenth days and Dale reactions were attempted on uterine strips. These tests were negative or so minimal that proof was still insufficient for stating the animals were sensitive. However, on further reading the statement was made that guinea pigs receiving multiple large doses could not be shocked for a period of six to eight weeks and the Dale reaction required three weeks to become positive.[2] Knowing this, but rather than waiting another three weeks for the sensitization of an additional group of virgin guinea pigs, ragweed pollen was insufflated into the other guinea pigs which had been sensitized at the same time as the original six virgin guinea pigs. On the twentieth and twenty-first days pollen was insufflated and the guinea pigs were sacrificed at twenty-four and thirty-six hours.

All animals were autopsied and a smear was made by stroking a glass slide over the viscera and peritoneal surface of the abdominal wall. These were dried, fixed and stained with Wright's stain. Sections were made of the peritoneal surface of the abdominal wall, and any other organs to which pollen was adherent. In addition sections were made of the liver, spleen, adrenals, lungs and thyroid. The smears made of the peritoneal fluid consistantly showed only epitheal cells and inflammatory cells. No eosinphils were noted on any smears. In the run down of the pathology of the rats there was no difference noted between the sensitive and the non-sensitive animals.

At the end of thirty minutes there is no evidence of inflammation grossly. There are scattered clumps of pollen collected over the peritoneal surface of the abdominal wall. Large masses are found in the omentum and small scattered clumps in the mesentery and over the surface of the intestines and liver. Microscopic examination reveals the adrenal, spleen, liver and thyroid to be normal. Pollen grains collected in the omentum show an occasional area of lymphocytes and leukocytes adjacent to the pollen. There is no evidence of any reaction to pollen collected on the surface of the liver. The lungs are essentially normal.

At one hour the gross pattern is similar to the pattern at thirty minutes, however, the pollen clusters are more firmly attached to the surface on which they are adherent. Microscopically the only difference noted is a beginning infiltration of leukocytes

leukocytes and lymphocytes. The blood vessels surrounding these areas of pollen appear congested. The pollen attached to the serosa of the bowel shows no evidence of reaction.

At two hours there is no difference in the reaction of the lungs, thyroid, liver, adrenal and spleen. Pollen grains scattered over the surface of the liver, intestine and peritoneal surface of the abdominal wall still show no sign of reaction. In the omentum, however, a considerable number of polymorphonuclear neutrophils are beginning to appear. There is no gross evidence of any reaction.

Three hours there is no gross difference from the two hour specimens. Microscopically more inflammatory reaction is seen about all the pollen clusters with the exception of that on surface of the liver. Some areas on the serosa of the intestine show a beginning leukocytic infiltration while others show no evidence of this. The lungs in all these animals show some congestion and recent hemorrhage. This however may be due to the manner in which the animals were killed. This was done by a sharp blow at the base of the skull.

At the end of four hours there is no gross evidence of inflammation. The lungs, adrenal, spleen and thyroid appear normal microscopically. The liver shows a normal architecture but the blood vessels appear slightly congested. Inflammatory cells are beginning to appear on the surface of the liver

adjacent to the pollen grains. The pollen granules seem to be imbedded deeper in the fatty tissue of the omentum with an intense infiltration of polymorphonuclear leukocytes. The blood vessels surrounding these areas appear dilated with some edema of the tissues. The inflammation surrounding the pollen on the peritoneal surface of the abdominal wall extends into the adjacent muscle. There were no eosinophils noted in this group of animals which is also true throughout the rest of this series.

At the end of six hours loose adhesions are present between loops of bowel, the omentum and the peritoneal surface of the abdominal wall at the sight of the insufflation. No other evidence of inflammation could be seen grossly. Lung, spleen, adrenal and thyroid appear normal. At this time, there is an acute inflammatory reaction where the pollen is attached to the liver surface. Sections are made through the area of adhesions. This shows the omentum to be adherent with leukocytes scattered throughout the fatty tissue of the omentum. In some areas the pollen seems to be surrounded by lymphocytes. These areas show a much less severe reaction.

At seven hours adhesions are apparent between loops of bowel, anterior abdominal wall, omentum and liver. These can be broken very easily, but with care one can lift almost the entire content of the abdominal cavity by picking up a single loop of bowel. There is still no other evidence of inflammation seen grossly. Microscopically the pollen now can be seen to be covered by a

thin film of fibrin. All masses of pollen now seem to be surrounded by an acute inflammatory reaction with edema of the associated tissue. Some pollen grains show a variation in staining. Other tissues appear normal.

In eight hours adhesions are still the only evidence of inflammation grossly. In these animals the reaction about the pollen is very variable. Some regions still show an intense inflammatory reaction while others show a much milder reaction. There is still edema of the tissues surrounding the reaction. Sections of other tissues are apparently normal.

At the end of twelve hours there is no noticable gross difference from the eight hour. Microscopically the reaction about the clumps of pollen are more variable than before. Some sites show an intense inflammatory reaction with edema of the surrounding tissue. Other areas show very little reaction, with all types of reaction in between. Other tissue sections are normal.

At twenty hours the adhesions between omentum, abdominal wall and loops of bowel appear firmer. Otherwise there is no gross difference from the twelve hour specimens. Microscopically the reaction still shows acute inflammation with edema and patchy leukocytic infiltration. There is less variation in the reactions surrounding the pollen in this group. Lungs, liver, spleen, adrenal and thyroid appear normal.

Grossly at twenty-four hours there is no difference from the twenty- hour specimen. Microscopically the edema of the tissue

surrounding pollen clusters seem more pronounced. The lungs of one of these animals shows many grains of pollen scattered throughout the blood vessels with beginning organization. This is interpretated as being embolic at the time of the intraperitoneal insufflation. Other sections of the tissue appear normal.

At twenty-eight hours, the clumps of pollen are more firmly adherent to the surfaces to which they are attached requiring a rather heavy stroke with an instrument to break the attachment. Otherwise the gross findings are the same as before. The microscopic examination shows a marked tendency towards walling off of the masses of pollen with strands of fibrin. There is still considerable edema of the surrounding tissues. The inflammatory reaction appears to be in older stage than in the previous animals. The pollen appears to be undergoing early signs of degeneration in some areas. Other tissues are normal.

At the end of thirty-two hours the gross pattern shows no apparent change from that noticed in the earlier specimens. Tissues not involved in the inflammatory reaction surrounding the pollen appear normal. Large numbers of large mononuclear cells are beginning to appear among the polymorphonuclear leukocytes surrounding the pollen. There is still a marked edema in the tissue surrounding the reaction. The walling off process by fibrin strands is also more apparent.

At this time, thirty-six hours, there is no gross difference than there has been in the previous twelve hours. Microscopically

the picture is essentially the same as at thirty-two hours. Throughout the whole series it has been rather difficult to differentiate a very marked change between sections of the previous time period. However, by studying slides with several time periods between, a marked change can be seen. This is in my opinion a very good evidence that the reaction is a slowly progressive one.

In the guinea pigs, table #3, the reactions noted are so similar to the reactions of the rats at the same period of time that no space will be relegated to describe their appearance.

Autopsy reports on the rats that died in shock which were mentioned earlier:

Rat #8 The lungs show considerable dilatation of the bronchi with inflammation and fibrosis of the bronchial wall. Occasionally a purulent exudate is present in the lumen. There are a few pollen grains present in the peritoneal surface of the abdominal wall. These are surrounded by a fibrous tissue proliferation. Other organs appear normal.

Rat #7 The only tissue which shows a deviation from the normal is the lungs. Here there is a dilatation of the bronchi with considerable lymphocytic infiltration.

Rat #2 The lung shows considerable dilatation of the bronchi with considerable lymphocytic infiltration about them. Sections through the sites of attached pollen masses show a granulomatous type lesion with fibrosis and giant cell formation. The pollen is undergoing degeneration.

Rat #3 Sections were made of the tissues of pollen collected over the surface of the bowel, liver and peritoneum of the abdominal wall and in the omentum. These show a granulomatous type lesion with fibrosis and giant cell formation less marked than in rats #8 and #2. The degeneration of pollen is more pronounced than it is in the aforementioned rats.

Rat #1 Sections here were very similar to those of #3. However, there are more foreign body giant cells and fibroblasts about the masses of pollen. The lungs show a chronic bronchitis.

Rat #5 and Rat #19 reveals granulomatous type lesion surrounding the degenerating pollen which is essentially no different than that described in the previous rats.

In the reports of the post mortem findings no attempt was made in this paper to differentiate between the sensitive and the non-sensitive animals. In the opinion of Dr. C. A. McWhorter, who studied the sections and prepared the pathology reports, there was no demonstrable difference.

Therefor, it is my conclusion that the peritoneum is a non allergic membrane for certainly one would expect a marked difference in the reactions noted between a sensitive and a non-sensitive animal. Since the normal pathology of allergy is a congestion of the blood vessels and a marked edema of the tissues. Whereas the findings described in these animals is the reaction one would expect to find with any innocous foreign body. This study of the reaction around the pollen also would tend to prove that the pollen gradually

degenerates until it is completely absorbed and disappears. There has been some additional work to prove this. Five rats were insufflated with pollen and killed after a period of five months. These failed to show any evidence of remaining pollen, either grossly or microscopically.

Additional work must be done to prove conclusively that animals can be sensitized by the method outlined in this paper. This work will be done in the near future but time prevents its inclusion in this paper.

## SUMMARY

1. Animals can be sensitized either by the injection of an extract of pollen intradermally or by the insufflation of pollen intraperitoneally.
2. There is no demonstrable difference in the reaction of ragweed pollen insufflated intraperitoneally in the sensitive and the non-sensitive animal.
3. Pollen which is trapped in the body sets up an inflammatory reaction which is gradually replaced by a granulomatous type of reaction. The pollen is walled off and gradually absorbed.
4. Since there is no difference in the reaction between the sensitive and the non-sensitive animals, I think it is safe to conclude that the peritoneum is a non-allergic membrane.

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