IMMUNOLOGICAL STUDIES IN RELATION TO THE SUPRARENAL GLAND

V. THE EFFECT OF REPEATED INJECTIONS OF SOLUTIONS CONTAINING SODIUM SALTS AND GLUCOSE ON THE HEMOLYSIN FORMATION OF NORMAL AND SUPRARENATECTOMIZED RATS

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(Received for publication, May 4, 1929)

In previous studies (1) on hemolysin formation in normal adult albino rats, it was found that 20 normal rats yielded an average titer of 1/7000, while in 23 additional normal rats the average titer was 1/4323. The average titer of the 43 rats collected in both series was 1/5656, somewhat lower than had previously been estimated with half the number of animals. Seventy per cent of these rats gave titers above 1/2000. An occasional very low reading was noted. From an analysis of the factors responsible for this variation it was observed that the females yielded somewhat higher hemolysin titers than the males (females 1/6850, males 1/4620). Older rats gave a higher antibody titer than young adult rats. Rats of greater weight but of the same age and sex yielded higher titers.

All the rats used in these experiments were bred in the laboratory under the same environmental conditions and were fed on a constant adequate diet. The variations observed in these animals in spite of these constant factors emphasize the necessity of maintaining the factors of age and weight constant and of utilizing large numbers of animals in studies on antibody formation. In our later studies only rats about 3 months of age were used and the results were more uniform.

It was found in previous experiments (2) that suprarenalectomy is followed by a depression in antibody forming capacity most marked during the first week following the operation. Further studies on hemolysin formation in this early period confirmed this observation. Of a series of 23 suprarenalectomized rats injected on the third day
following the operation with 1 cc. of a 10 per cent suspension of sheep cells and tested 5 days later, the average hemolysin titer was 1/858 as compared with 1/5650 of the normal rats. About 70 per cent of suprarenalectomized rats gave titers below 1/2000. Two rats gave high titers corresponding to normal figures. This may be explained by the well known fact that a certain small percentage of suprarenalectomized rats react as normal rats, due to large amounts of accessory cortical tissue (3). Of the suprarenalectomized rats, as of the normal rats, the females gave slightly higher titers than the males.

In the course of experiments on the effect of injections of epinephrine on the hemolysin formation in suprarenalectomized rats (4) it was noted that repeated injections of epinephrine in amounts of 0.2 mg. per kilo raised the hemolysin titer. Small amounts of physiological salt solution raised the titer to about the same level as the epinephrine injected group. This observation suggested the experiments reported in this communication. Studies on hemolysin formation were made in suprarenalectomized rats injected repeatedly with large quantities of a 0.9 per cent salt solution, isotonic sodium acetate, isotonic glucose solution and hypertonic salt solution in order to determine the effect of these substances on antibody production.

Methods

The method of operation, the post-operative care of the animals and the exact procedure of obtaining the blood and titrating the serum has been described in detail in previous communications (5) (2).

In all the experiments reported in this paper, 1 cc. of a 10 per cent suspension of sheep cells was injected intraperitoneally and titers were studied on the 5th, 8th and 11th day following the injection. When suprarenalectomized rats were studied the antigen was injected 48 to 72 hours after operation and the first titer was read on the 7th or 8th day after suprarenalectomy.

The Effect of Injections of Physiological Salt Solution on Hemolysin Formation in Normal and Suprarenalectomized Rats

Four groups of rats were tested. One group of 26 normal rats received two daily injections of 0.5 cc. of physiological salt solution during 3 days prior and 4 days subsequent to the injection of antigen. The average titer in these rats was 1/5516 or about the same as untreated normal rats. A second group of six normal rats
received 5 cc. of physiological salt solution intraperitoneally, once daily during 3 days prior and 4 days subsequent to the injection of sheep cells. The average titer was 1/4133, slightly lower than the titer of normal control rats.*

A third group of thirteen suprarenalectomized rats received 0.5 cc. of physiological salt solution subcutaneously twice daily. The average titer in this group was 1/2630 as compared with 1/858 of the untreated suprarenalectomized rats.

A fourth group of ten suprarenalectomized rats received 5 cc. of physiological salt solution intraperitoneally once daily from the day of operation to the day of the last reading. The average titer in this group was 1/3580, strikingly higher than the titer of untreated suprarenalectomized rats. The general condition of suprarenalectomized rats that had received salt solution injections was excellent. They ate well, exercised vigorously, kept their fur white and sleek and appeared stronger than the uninjected rats. These results indicate a marked beneficial effect from repeated injections of 0.9 per cent salt solution on the general well being and antibody forming capacity of suprarenalectomized rats.

Effect of Injections of Isotonic Sodium Acetate Solution on the Hemolysin Formation of Suprarenalectomized Rats

In an effort to determine the factors responsible for the rise in hemolysin titer in suprarenalectomized rats injected with physiological salt solution, other solutions were used. Isotonic sodium acetate solution was injected into ten suprarenalectomized rats in the same quantities by volume and over the same period of time as those injected with physiological salt solution. The average titer was 1/4260 or about five times as high as the untreated suprarenalectomized rats (1/858). It is apparent therefore that the chlorine ion is not essential

* Belak and Csereszynes (6) found some stimulating effect of physiological salt solution on the agglutinin formation following the injection of paratyphoid B bacilli in normal rabbits. They injected 0.5 cc. of the solution daily for 14 days after the injection of the antigen. Ringer's solution and isotonic calcium chloride had the same effect. They do not believe the sodium ion was responsible for the rise. Their data were collected on four rabbits. We were unable to demonstrate analogous findings in the case of hemolysin formation in normal rats.
in raising the antibody titer in suprarenalectomized rats. Normal control rats injected with isotonic sodium acetate solution in amounts of 5 cc. per day during 3 days prior and 4 days subsequent to the injection of sheep cells yielded titers slightly lower than untreated normal rats.

**Effect of Repeated Injections of Isotonic Glucose Solution on the Hemolysin Titer of Suprarenalectomized Rats**

To determine whether the effect of salt solution on the antibody formation in these rats was due to its diuretic effect, 5 cc. of isotonic glucose solution was injected once daily intraperitoneally into eight suprarenalectomized rats during the entire period from the day of operation to the day of the last reading of the hemolysin titer. The average titer of this group was 1/887, or about the same as that of untreated suprarenalectomized rats. In spite of its diuretic action, isotonic glucose did not raise the antibody formation of suprarenalectomized rats. In a group of normal control rats which were injected with isotonic glucose solution there was almost no change in the hemolysin titers from that of uninjected normal rats. The average titer in this group was 1/4500.

**The Effect of Concentrated Solution of Sodium Chloride on the Hemolysin Formation of Suprarenalectomized Rats**

An effort was made to determine whether sodium chloride alone had the same effect as large volumes of isotonic salt solution. 0.5 cc. of a 10 per cent solution of sodium chloride was injected into eight suprarenalectomized rats once daily from the day of operation during 3 days prior and 4 days subsequent to the injection of sheep cells. The hypertonic solution was extremely irritating. It was observed that the concentrated salt solution had only a slight effect in raising the antibody formation in suprarenalectomized rats. The average titer in this group was 1/1660.

**DISCUSSION**

It is apparent from these data that injections of isotonic solutions of sodium salts raise the titer of suprarenalectomized rats. This rise in hemolysin titer is not due entirely to the diuretic effect of the large
volume of salt solution injected, since the same volume of glucose solution, an excellent diuretic, has no such effect. It was found that sodium chloride administered in a small volume of fluid but in the same amount by weight as in the isotonic solutions has only a slight effect in raising the titer in suprarenalectomized rats. It would seem from these observations that both the fluid and the sodium ion are necessary to bring about a restoration of the antibody forming capacity of suprarenalectomized rats to normal.

Our results are in accord with the findings of Marine and Baumann (7). They found that the administration of Ringer’s solution and isotonic solutions of sodium chloride and sodium acetate increased the duration of life of suprarenalectomized cats about three times. More concentrated solutions of sodium chloride definitely shorten life. Isotonic solutions of glucose and glycerol had only a very slight life-prolonging effect. They concluded from their studies that diuresis is one of the important factors in determining the duration of life. When the loss of water by diuresis is compensated for by an additional intake, life is prolonged and if not, life is shortened. There is some indication, they believe, that the loss of sodium is more specific than can be accounted for as a result of a possible acidosis.

It is well known that the sodium ion plays an important rôle in the water exchange in the tissues. Suprarenalectomy results in a profound disturbance of the water balance in the body. Following the removal of the glands, there is a loss of fluid through the intestines, a diminution of sodium concentration in the blood (Baumann and Kurland (8)) and an impairment of kidney function. These factors result in tissue dehydration and a disturbance of the normal cellular physiological activity. Those tissue elements concerned with antibody production are less able to respond to the stimulation of foreign substances with the production of immune bodies in suprarenalectomized rats than in normal rats. There may, however, be no actual loss of immune body production but a diminished release of these antibodies into the blood stream. The restoration of the water balance by the promotion of diuresis with a simultaneous, increased intake of fluid in a form that can be retained in the tissues helps to restore the mechanism of antibody production to the normal. The rôle of the suprarenal gland in antibody formation appears to be intimately connected with the maintenance of water balance.

The relation of a disturbance in the water balance of the tissues to
### TABLE I

<table>
<thead>
<tr>
<th>Fluid injected</th>
<th>Route of injection</th>
<th>Amount per injection</th>
<th>No. of rats</th>
<th>5th day</th>
<th>6th day</th>
<th>7th day</th>
<th>8th day</th>
<th>9th day</th>
<th>10th day</th>
<th>11th day</th>
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<td></td>
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<td></td>
<td></td>
<td>Maximum</td>
<td>Minimum</td>
<td>Mean</td>
<td>Maximum</td>
<td>Minimum</td>
<td>Mean</td>
<td>Maximum</td>
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<tr>
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<td></td>
<td>21</td>
<td>3000*</td>
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<td>800</td>
<td>858</td>
<td>4000</td>
<td>60</td>
</tr>
<tr>
<td>isotonic sod. chlor.</td>
<td>subcut.</td>
<td>0.5 cc. twice daily</td>
<td>13</td>
<td>12000</td>
<td>10000</td>
<td>1000</td>
<td>2000</td>
<td>2630</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>&quot;</td>
<td>intrap.</td>
<td>&quot;</td>
<td>10</td>
<td>16000</td>
<td>10000</td>
<td>1000</td>
<td>3000</td>
<td>3580</td>
<td>600</td>
<td>40</td>
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<tr>
<td>isotonic glucose</td>
<td>&quot;</td>
<td>&quot;</td>
<td>8</td>
<td>8000</td>
<td>8000</td>
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<tr>
<td>&quot;</td>
<td>subcut.</td>
<td>0.5 cc. twice daily</td>
<td>10</td>
<td>16000</td>
<td>16000</td>
<td>1600</td>
<td>4000</td>
<td>4260</td>
<td>2000</td>
<td>3000</td>
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<tr>
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<td>&quot;</td>
<td>&quot;</td>
<td>10</td>
<td>8000</td>
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<tr>
<td>hypertonic sod. chlor.</td>
<td>&quot;</td>
<td>&quot;</td>
<td>7</td>
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</tbody>
</table>

* A single high reading of 16000 was omitted from average.
antibody formation has hitherto not been noted by previous investigators to the authors' knowledge. This observation is of importance in the interpretation of certain phenomena in acute infections. It is a matter of empiricism that "forcing fluids" is beneficial in the treat-

![Graph showing the effect of solutions containing sodium salt and glucose on the hemolysin formation of normal and suprarenalectomized rats. The columns represent the average hemolysin titer for each group.](image-url)
ment of severe infections and in extensive skin burns. It is known that in many acute infections, and especially in burns, the suprarenal
glands are severely injured. This injury in turn may result in a disturbance of the tissue water balance and in the antibody forming capacity of the body. This disturbance may be corrected to some extent by parenteral and oral administration of large quantities of physiological salt solution.

In their recent studies on experimentally produced peritonitis in dogs, Orr and Haden (9) injected 1 per cent solutions of sodium chloride and found that the animals lived three times as long as those not given the salt solution. This may be due to the effect of the salt solution on the mechanism of water metabolism disturbed as a result of the suprarenal injury which accompanies a general peritonitis.

**SUMMARY**

Table I and Figs. 1 and 2 summarize the experiments done on 150 rats. Suprarenalectomy in rats is followed by a striking depression in antibody formation most marked during the first week following the operation. Repeated injections of large amounts of isotonic sodium chloride or sodium acetate solutions restore the antibody titer to normal. The effect of these solutions is not due to diuresis alone, since isotonic glucose solution does not affect the titer, nor to the sodium alone, since hypertonic solutions of sodium chloride in small volume have little influence on the titer. Both the sodium ion and the water volume are necessary in the restoration of the titer.

**CONCLUSION**

(1) Suprarenalectomy is followed by a disturbance in the antibody forming mechanism of the body. (2) The suprarenal gland plays a rôle in the water metabolism of the tissues. (3) Restoration of the water exchange in the tissues to a normal level is sufficient to raise the antibody forming capacity to normal.

We are indebted to Dr. David Marine for his helpful suggestions in the course of this work.

**BIBLIOGRAPHY**


