OBSERVATIONS ON THE INFLUENCE OF THE HYPOPHYSIS AND THE ADRENAL CORTEX ON BLOOD PLATELET LEVELS

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THE MAMMALIAN blood platelet remains the formed blood element about which there is little significant information. Major areas of ignorance include the factors concerned with the regulation and the mechanism of platelet formation, release, and utilization, as well as with the exact role of platelets in vascular hemostasis and plasma coagulation. The observations described in this paper were planned to examine the hypothesis that the circulating level of blood platelets might be subject to the influence of certain endocrine secretions. Although no clear indication for such a relationship exists, several lines of evidence are consistent with this working hypothesis. First, a variety of stressful stimuli, including fever, severe exercise, anoxia, hemorrhage, trauma and surgery are reported to result in significant elevation of the platelet count. Since such conditions have as one common factor the stimulation of the pituitary-adrenal cortex system, it seemed reasonable to evaluate the possibility that these glands exercise a direct influence on the mechanisms determining the level of circulating platelets.

Secondly, much recent work has revealed a relationship between the activity of several endocrine glands and processes of hematopoiesis involving both the red and white cell series. The anemia which follows hypophysectomy and the control of lymphocytes exerted by the pituitary-adrenal cortex system represent the more clearly established correlations between endocrine secretions and processes concerned with hematopoiesis.

Finally, there is some evidence that hemostatic vascular reactions, believed to involve the blood platelets, may be altered by endocrine influences. Ungar presented data indicating that the spleen, activated by the pituitary and the adrenal cortex, secretes a substance effective in shortening bleeding time and increasing capillary resistance.

Observations directed specifically toward a possible endocrine influence upon platelet levels are few in number. Estrogens, administered in massive doses over a period of weeks, have been reported to reduce the platelet counts of dogs and monkeys to purpuric levels, the final picture being that of an aplastic anemia in which all the cellular components, both of the peripheral blood and bone marrow, were at low levels. Sheket and associates observed an average increase of 76 per cent in the blood platelets during the terminal postoperative week in adrenalectomized rats. A report by Dalton, Masson and Selye describes a similar steady rise in platelets following bilateral adrenalectomy in rats. The results of sham
operation are mentioned in neither report, however, and the well-substantiated phenomenon of a platelet elevation following all types of surgery\(^1\) makes it impossible to ascribe specifically to the absence of the adrenals the platelet changes reported in these two studies. Zondek and Kaatz\(^2\) reported a moderate reduction in the platelets of men one hour following the administration of small doses of adrenal cortex extract (Richter—cortigen), and an increase following oral thyroid and parenteral thyroxin or thyrotrophic hormone. Following castration of male and female albino rats a significant drop in platelets lasting several months has been described, as well as a gradual elevation of platelets following the subcutaneous injection of gonadal extract.\(^3\)

**Materials and Methods**

The animals used in this study were male Sprague-Dawley rats weighing between 175 and 300 Gm., male and female mice of both the A and the CBA strain, between 8 and 12 weeks of age, and young adult male rabbits of mixed strain. All animals were kept in air-conditioned rooms at controlled temperatures and fed a standard diet composed of Purina Lab Chow (rats and mice) or Purina Rabbit Chow.

The platelet counting method employed is described in detail elsewhere.\(^4\) In brief, the method for rats and mice was as follows: By heart puncture a small standard quantity of blood (0.1-0.2 cc.) was aspirated into a 2-cc. syringe containing a measured volume of sodium oxalate solution. A quantitative dilution of the blood having been made in the original syringe, the contents were mixed, transferred to a test tube and allowed to remain undisturbed until a clear layer of diluted plasma appeared as a result of the sedimentation of erythrocytes and leukocytes. This layer was then sampled with a capillary pipet and the platelets counted in a hemocytometer of conventional type. The method for rabbits was identical in essentials except that blood was drawn from the ear artery rather than from the heart. In the case of mice, it was necessary to sacrifice an animal for each determination, the heart puncture being performed after opening the chest. Serial counts could be easily made both in rats and rabbits.

Splenectomy and bilateral adrenalectomy were performed on rats and mice in the usual manner, under ether anesthesia, using a clean but not sterile technic. Following bilateral adrenalectomy mice were given routinely a single subcutaneous injection of desoxycorticosterone acetate in sesame oil (0.2 cc. containing 1.25 mg.); both rats and mice were given a 1 per cent solution of NaCl as drinking water following adrenalectomy. In many, but not all cases, completeness of adrenalectomy was checked by autopsy.

Male, Sprague-Dawley rats, hypophysectomized at about 1 months of age, were obtained from the Hormone Assay Laboratory, Inc., Chicago. Hypophysectomy was considered complete if the animals failed to gain weight, and if a marked degree of testicular atrophy appeared.

Aqueous adrenal cortex extract (Wilson) was the preparation of cortical hormone used, and was administered to rats subcutaneously in doses of 1 cc. per 100 gm. body weight. Injected control fluids such as physiologic saline and water, were given in the same doses. Mice received 0.25 cc. of aqueous adrenal cortex extract subcutaneously; rabbits were given 10 cc. of this preparation subcutaneously.

**Results**

*Adrenal Cortex Extract in the Intact Animal*

Large single doses of aqueous adrenal cortex extract were found to be without influence on the platelet counts of mice, rats and rabbits. Mice (CBA strain) were sacrificed in groups of 4 to 10 individuals at intervals from fifteen minutes to forty-eight hours following hormone. A group of 16 rats was subjected to platelet counts immediately before, three and twenty-four hours after the injection of adrenal cortex extract. Six rabbits were followed with serial platelet counts at intervals of 1, 4, 8, and 24 hours after hormone. At no interval following injection in any
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Fig. 1.—Serial changes in the platelet count following adrenalectomy: each point represents the percentage change from the last pre-operative count in a given rat.

Fig. 2.—Serial changes in the platelet count following sham-adrenalectomy: each point has same significance as in figure 1. Note similarity to the response after adrenalectomy in figure 1.

of these species, could consistent, significant differences from the control values be detected.
Adrenalectomy

Platelet counts were performed serially following adrenalectomy in a group of 25 male Sprague-Dawley rats, and following sham-adrenalectomy in a group of 19 similar animals. Both types of operation were carried out in identical fashion, except that in the control operation a piece of perirenal fat was removed from the vicinity of each kidney without disturbing the adrenal gland. The similarity of the platelet response, both in magnitude and duration, can be seen from inspection of figures 1 and 2. In these figures, each point represents the calculated percentage difference between the platelet count on a particular postoperative day in a given animal, and the preoperative count in the same individual. Additional data, covering only the first five postoperative days, revealed no significant difference in the platelet rise following adrenalectomy and sham-adrenalectomy in mice of the A strain.

Adrenal Cortex Extract Following Adrenalectomy

In contrast to the failure of adrenal cortex extract to influence the platelet level of intact rats, comparable doses of hormone given to rats following adrenalectomy were found to produce a consistent reduction in the platelet count as detected by comparing counts made in the same animal immediately before and three hours after administration. Individual values for the per cent reduction in platelet numbers ranged between 9 per cent and 18 per cent, the consistency of the direction of change being more striking than its magnitude. Rats were first examined for platelet changes following hormone administration four or five days after adrenalectomy, and the same rats, in most instances, were used for repeat observations at longer periods—between ten and sixty days—postoperatively. A control group of rats subjected to sham-adrenalectomy and examined five to eleven days following operation, was found to show no consistent platelet response to adrenal cortex extract. It should be mentioned that the group of rats originally used to examine the effects of adrenal cortex extract on platelets in the intact animal was subsequently subjected to adrenalectomy or sham-adrenalectomy for the postoperative trial of hormone, so that with a few exceptions, the same group of individual rats served both as control and experimental animals.

In some, but not all, instances, autopsy was performed on rats allowed to survive for long periods (up to sixty days) after adrenalectomy. In a few of these animals regenerated adrenal tissue was found, and the data discarded. No animal was used in the adrenalectomy series unless removal of both adrenals intact had been accomplished. It was considered that adrenalectomy was functionally complete at the five-day interval, even though adrenal regeneration might have occurred many days subsequently. No adrenal tissue was found in any of a group of 13 rats autopsied five to seven days following adrenalectomy.

In an attempt to control these observations further, comparable doses of physiologic saline were given subcutaneously to a group of adrenalectomized rats on the fifth postoperative day. The unexpected finding was made that in the adrenalectomized, but not the sham-operated rat, this treatment too was followed by a
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fall in platelets comparable in magnitude to that observed after administration of adrenal cortex extract. In contrast, distilled water given in similar quantity to a group of adrenalectomized rats was succeeded by no significant change in the platelet count. These data are summarized in table 1.

The unlikely possibility that a reduction in the platelet count of such magnitude might be ascribed to hemodilution in the adrenalectomized rat, brought about by the actual volume of adrenal cortex extract or saline injected, was tested by following the change in hemoglobin* concentration three hours after the subcutaneous administration of physiologic saline to a small number of rats five days after adrenalectomy or sham-adrenalectomy. Under these conditions, the maxi-

Table 1.—Comparison of average percent change in the platelet count immediately before and 3 hours after the administration of several different preparations to intact, adrenalectomized and sham-adrenalectomized rats

<table>
<thead>
<tr>
<th>Operative Group</th>
<th>Material Injected</th>
<th>Number of Animals</th>
<th>Days Postoperative</th>
<th>Percent Change in Platelets 3 Hours After Injection*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>Adrenal Cortex Extract</td>
<td>16</td>
<td></td>
<td>+4 ± 1.6</td>
</tr>
<tr>
<td>Sham Adrenalectomy</td>
<td>Adrenal Cortex Extract</td>
<td>10</td>
<td>5-11</td>
<td>0 ± 2.1</td>
</tr>
<tr>
<td>Adrenalectomy†</td>
<td>Adrenal Cortex Extract</td>
<td>16</td>
<td>4-5</td>
<td>-17 ± 2.3</td>
</tr>
<tr>
<td>Adrenalectomy†</td>
<td>Adrenal Cortex Extract</td>
<td>12</td>
<td>10-60</td>
<td>-11 ± 3.1</td>
</tr>
<tr>
<td>Sham Adrenalectomy</td>
<td>Saline</td>
<td>10</td>
<td>5-7-13</td>
<td>-2 ± 2.2</td>
</tr>
<tr>
<td>Adrenalectomy</td>
<td>Saline</td>
<td>7</td>
<td>5-7</td>
<td>-18 ± 3.0</td>
</tr>
<tr>
<td>Adrenalectomy</td>
<td>Water</td>
<td>6</td>
<td>5</td>
<td>+1 ± 1.6</td>
</tr>
</tbody>
</table>

* Means and standard errors.
† Values significantly (p < 0.01 by t-test) lower than all other mean values shown. No significant differences between any other two sets of means.

maximum reduction in hemoglobin was 7 per cent below the preinjection level, the mean for five animals being a fall of 4 per cent.

Hypophysectomy

Male Sprague-Dawley rats hypophysectomized at about 2 months of age were followed with serial platelet counts. All counts made within a three-week period after hypophysectomy were not included in analyzing the data, because of possible nonspecific effects on the platelet level of the operation itself. As can be seen from table 2, the average value for a group of platelet counts in 23 hypophysectomized rats was significantly, although not strikingly, lower than the average for a group of 71 intact rats of the same sex, strain and approximate age.

* Hemoglobin was determined in the Coleman, Jr., Spectrophotometer by the alkaline hematin method.23
The phenomenon of a reduction in platelet numbers following the administration of adrenal cortex extract to adrenalectomized rats might suggest the possibility of a similar change in hypophysectomized rats after a postoperative interval sufficient to permit adrenal atrophy. No significant difference was noted, however, between the preinjection and three-hour postinjection platelet counts of a group of 6 rats given subcutaneous adrenal cortex extract twenty-eight days after hypophysectomy.

| Table 2. — Average platelet values in intact and hypophysectomized rats |
|--------------------------|----------|---------------------|
|                         | Number of Animals | Number of Counts | Platelets/mm³, blood (x 1000)* |
| Intact                  | 71        | 188                 | 989 ± 14.8                   |
| Hypophysectomized       | 23        | 47                  | 854 ± 20.4                  |

* Means and standard errors.
† Significantly lower than control mean (p < 0.01 by t-test).

Platelet Response to Splenectomy in Intact and Hypophysectomized Rats

Of all types of surgery, splenectomy is generally followed by the largest and most enduring postoperative elevations in the platelet count. There is some evidence that this phenomenon is due to removal of the large complement of reticuloendothelial cells in the spleen, which may normally play a role in clearing platelets from the circulation. An alternative explanation holds that the spleen normally exerts an inhibitory effect on platelet formation in the bone marrow, an activity quite clearly demonstrated by Dameshek and Miller in patients with essential thrombocytopenic purpura.

In contrast to the minimal reduction of the platelet count as a result of hypophysectomy, the platelet response following splenectomy was found to be markedly depressed in the hypophysectomized rat as compared with the effects of splenectomy in the intact rat.* On the fifth and sixth day postsplenectomy in the otherwise intact rat, maximum platelet levels were observed, representing percentage increases roughly 100 per cent above the preoperative level. At a similar interval after splenectomy in hypophysectomized rats, increases averaging about 40 per cent were noted. These data are expressed in figures 3 and 4, in which each point plotted represents the calculated percentage difference between the platelet count in a given rat at the indicated postoperative level and the preoperative count in the same animal.

A small number of intact and hypophysectomized rats, before and five days after splenectomy, were autopsied to provide marrow specimens examined by the method described by Mayer and Ruzicka. This technic permits the microscopic examination of a longitudinal section of the entire femoral bone marrow fixed in

* Most of the hypophysectomized rats were subjected to splenectomy at least 1 month following hypophysectomy. In 4 rats, splenectomy was performed only eight days following hypophysectomy: no difference in platelet response was observed in this group as compared with the results of splenectomy in the remaining hypophysectomized animals.
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Fig. 3.—Platelet response to splenectomy in the intact rat: each point represents the percentage change from the last preoperative count in a given rat.

Fig. 4.—Platelet response to splenectomy in previously hypophysectomized rats. Each point has the same significance as in figure 3.

situ, and was thought to be the best method available for enumerating bone marrow elements of infrequent occurrence such as megakaryocytes. The megakaryocytes seen in 20 high-dry fields (magnification 400 diameters) of each bone mar-
row section were counted; the results, recorded in table 3, indicate a significant reduction of megakaryocytes in the hypophysectomized rat both before and after splenectomy, but no significant change in the number of megakaryocytes following splenectomy either in intact or hypophysectomized rats. The failure to note quantitative changes in megakaryocytes following splenectomy agrees with the observations of Higgins and Stasney that marrow imprints made following splenectomy in otherwise intact rats revealed no significant increase in the number of megakaryocytes, despite a large postoperative rise in circulating platelets.

Inspection of megakaryocytes in Giemsa-stained smears of the bone marrow of rats in the four categories cited above—intact and hypophysectomized, before and after splenectomy—revealed no qualitative morphologic differences in megakaryocytes, such as depression of platelet formation, absence of granularity, and other changes of the type described by Dameshek and Miller in idiopathic thrombocytopenic purpura of man.

<p>| Table 3.—Bone-marrow megakaryocytes in hypophysectomized and intact rats before and 3 days after splenectomy |
|-------------------------------------------------|------------------|------------------|</p>
<table>
<thead>
<tr>
<th>Relation to Splenectomy</th>
<th>Number of Animals</th>
<th>Number of Megakaryocytes*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>Before</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>3</td>
</tr>
<tr>
<td>Hypophysectomized†</td>
<td>Before</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>3</td>
</tr>
</tbody>
</table>

* Number per 20 high-power fields: Means and standard errors.
† Both hypophysectomized means significantly lower than either intact mean (p < 0.01 by t-test). No significant difference before or after splenectomy in either group.

Other relevant observations included changes in the weight and histologic appearance of spleens removed from hypophysectomized rats. As reported earlier by Perla, hypophysectomy is followed by a progressive reduction in spleen weight. In the present study, the spleen/body-weight ratio in rats a month after hypophysectomy was found significantly lower than the same value in intact rats of corresponding age. Histologic changes in the spleens of hypophysectomized rats included the presence of fewer megakaryocytes in this tissue as well as in the bone marrow, and a reduction of mitotic activity in the germinal centers of lymphoid follicles. A fuller account of the morphologic alterations in the spleen after hypophysectomy can be found in Perla's paper, which also describes hyperplasia of the germinal centers of splenic lymph follicles, and an increased number of megakaryocytes, both in spleen and bone marrow, in rats given extracts of dried beef pituitary.

Discussion

The results described do not support the hypothesis that blood platelets are influenced in any specific or significant way by the hormones of the pituitary or adrenal cortex. The almost exact similarity in response of platelets to sham opera-
tion and to adrenalectomy would seem to confirm the suspicion that those reports describing large increases in platelets after adrenalectomy, were simply observations of the well-known phenomenon of postoperative thrombocytosis seen after major surgery of almost any nature.

Of more positive interest, although difficult to relate to other findings, is the observation that the administration either of adrenal cortex extract or physiologic saline is followed by a significant fall in platelets in the adrenalectomized but not in the sham-operated rat. This finding, coupled with the failure of distilled water to influence the platelets of adrenalectomized rats, suggests a possible electrolyte effect, although no further light can be thrown on this question with the data of the present study. Whatever mechanism underlies this observation, the fact that it does not occur in rats some weeks after hypophysectomy suggests that it requires the absence, rather than a moderate relative functional insufficiency, of the adrenals.

Observations of platelet levels following hypophysectomy, and the platelet response to splenectomy of the hypophysectomized rat, raise some interesting questions as to the equilibrium of production and removal rates which must govern the level of circulating platelets. First, the small, if significant, decline in platelets in the hypophysectomized rat, does not by itself suggest any primary action of the pituitary on platelet levels. Changes of this magnitude might be considered part of the picture of generalized tissue atrophy and lowered tissue metabolism following hypophysectomy, just as the reduction of megakaryocytes in the marrow is part of the picture of generalized marrow hypoplasia.

The marked reduction in the thrombocytosis following splenectomy in the hypophysectomized animal, however, suggests an additional possibility. The atrophy of the spleen which occurs after hypophysectomy may quite possibly indicate a reduced functional capacity of this organ, and perhaps other reticulo-endothelial tissue, to remove platelets from the circulation. With such a reduction in level of both platelet-forming and platelet-removing potential, a new equilibrium in the level of circulating platelets might be established, which would not differ greatly from the level in the intact animal. Sudden removal of a large component of reticulo-endothelium, as by splenectomy, might then temporarily unmask the reduced production capacity (by eliminating the balancing factor of platelet removal), and permit its detection in terms of a much depressed platelet response to splenectomy. Such an explanation is of course not uniquely determined by the observed facts, but merely fits them with reasonable simplicity.

**Summary**

Observations were made to investigate possible endocrine influences on blood platelets. Adrenal cortex extract failed to influence the platelet counts of mice, rats, or rabbits. Adrenalectomy and sham-adrenalectomy were followed by almost identical platelet increases in mice and rats. Administration of adrenal cortex extract, or physiologic saline, to adrenalectomized rats was followed by a consistent fall in platelets not observed in sham-adrenalectomized rats, or after administering distilled water to adrenalectomized rats. Platelet levels in hypophysectomized rats were significantly lower than in unoperated controls. Splenec-
Platyctomy in hypophysectomized rats was followed by a maximum rise in platelets markedly lower than following splenectomy in intact rats. Bone-marrow megakaryocytes in hypophysectomized rats were significantly fewer than in intact rats. No changes in megakaryocyte number or morphology appeared following splenectomy either in intact or hypophysectomized rats.

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REFERENCES
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