The Influence of the Suprarenal Gland on the Thymus.

I. Regeneration of the Thymus Following Double Suprarenalec-tomy in the Rat.

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Plate 13.

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The purpose of this paper is to present experimental evidence showing the close interrelation between the thymus and suprarenal glands, our results being based upon a study of 66 doubly suprarenalec-tomized rats and 21 non-operated controls.

No detailed and convincing experimental literature is to be found concerning thymic enlargement following suprarenal injury or ablation. Mention of such thymic enlargement has always been re-port ed incidentally and, in most cases, the findings are open to severe criticism.

During the last 30 years very frequent mention has been made in clinical and pathological reports of the association of a large thymus and diseased suprarenals. With the accumulation of data upon this disease, the association becomes more constant and significant. Addison (2), in his classical monograph, does not de-scribe thymus enlargement in the disease which now bears his name, nor does Averbeck (3), who collected, in 1869, 61 cases from the literature. Star (4) was the first to call attention to thymic enlargement in Addison's disease, in the case of a girl, 17 years of age, who died suddenly, and who at autopsy showed a large thymus associated with diseased suprarenals. The more comprehensive reports of Wiesel (5), Hedinger (6), Hart (7), and Pappenheimer (8) soon after definitely established what Star had noted, the association of thymic enlargement with diseased suprarenals.

Experimentally, on the other hand, Boinet (9) and Calogero (10), using rats, and Auld (11), working with cats, have referred to thymic enlargement following suprarenalec-tomy. Auld reports large thymuses in four cats which died within
2 days following the removal of the second suprarenals, the first glands having
been removed some months previously. While removal of one gland in the cat
may induce some degree of suprarenal insufficiency, it is doubtful whether the
insufficiency thus induced could have been the causative factor producing the
large thymuses noted by Auld. Also there could have been no significant enlarge-
ment during the 2 days following removal of the second glands, as this time is
insufficient for hyperplastic changes to manifest themselves.

Calogero's rats also died within 2 days following suprarenelectomy. Further-
more, he knew neither the age nor the life history of his animals, he gave no weights,
nor did he have proper controls, so that his conclusions in regard to thymus en-
largement are open to severe criticism. The apparent enlargement he reports
could be attributed, for example, to age differences of his animals.

Boinet reported, among other changes, thymus enlargement in eleven rats of a
series of 59 which he suprarenelecomized, many of which survived bilateral
suprarenal extirpation for some weeks. Like Calogero, he knew neither the exact
age nor the life history of his animals, nor was his work properly controlled; he did
not give the weights or measurements of the glands which he believes were enlarged.
Since he reports thymus enlargement in some animals which died within 1 and 2
days following suprarenelectomy, and, as was pointed out previously, this is
physiologically improbable, his conclusions are also subject to the same criticisms
that were applied to Calogero's work.

Crowe and Wislocki (12), in 1914, while studying the effects of piecemeal supra-
renal ablation in dogs, reported enlargement of the thymus in four animals of a
series of twenty that survived, in good condition, subtotal suprarenal extirpation
for from 2 weeks to 6 months. These workers conclude that "the most striking
feature at autopsy on an animal with a long-standing adrenal insufficiency is
the enlargement of the mesenteric and retroperitoneal lymph glands and the
solitary lymph follicles in the walls of the intestine. Not infrequently there is
also an hyperplasia of the thymus." Their work was well controlled and their
findings with regard to the thymus, though they had only four animals, would
indicate that secondary hyperplasia of this organ will take place in dogs following
subtotal suprarenal ablation.

Recently, Aub (13) and his coworkers, studying metabolic changes after supra-
renalnectomy, incidentally mentioned enlargement of the abdominal lymph nodes
and thymus gland in their suprarenalecomized cats. Aub's animals died or were
sacrificed within a few days following double suprarenelectomy. It is important
to emphasize again the danger of drawing conclusions concerning hyperplasia of
the thymus in moribund animals since it is definitely established that involution-
ary changes rapidly take place in dying animals.

Marine and Baumann (14), and Také and Marine (15), have pointed out the
frequent occurrence of hypertrophy of the thymus and lymphoid tissues in rabbits
which survived the removal of both glands. The present work was undertaken
with the view of obtaining more specific and conclusive data on this point.
This work on the rat is the first in which thymic enlargement following suprarenalectomy has been observed and systematically studied in a large series of animals. By the use of operated and non-operated controls, and a comparison with the tables for the normal rat as compiled by Donaldson (16), we have demonstrated this change objectively. The rat is admirably adapted for such studies, in which a large number of animals is required, because a high percentage of these animals survive double suprarenalectomy and, above all, because they are highly resistant to pyogenic infection.

Methods.

All rats used were reared in the laboratory from very tame albino stock (Mus norvegicus albinus), obtained from the Wistar Institute. Wistar rats offer the advantage in that growth and developmental curves for the body as a whole, and for the various organs, have been tabulated.

The young are weaned at about 25 days of age and are then put on a completely adequate diet consisting of 15 parts rolled oats, 60 parts hominy, 14 parts dried meat scrap, 10 parts dried milk powder, and 1 part salt. This diet is supplemented with bread and fresh milk daily until the animals are 45 days of age, after which bread and milk are given once a week; fresh vegetables are fed two or three times a week and all rats are given fresh tap water daily. During the period of active growth the rats are placed in long cages. The animals are fairly free of snuffles, and chronic pneumonia is uncommon even in 1 year old rats.

Our operative technique was standardized; both glands were removed at one sitting and after induction; ether anesthesia was always used by the open method. The operative field was prepared by shaving, and this was followed by wiping area with sponges dipped in bichloride solution. At first the operations were done through a midline upper abdominal incision, but this was later abandoned since the method of removing the glands through the back route was found more satisfactory, yielding about 85 to 90 per cent recoveries in adult rats. Food was withheld for 18 hours before operation and the rats were not fed for 12 hours after operation. For 2 days following suprarenal ablation, bread and fresh milk only were given, then the dry food mixture and vegetables were added. The operated rats were kept for about 2 weeks in individual cages, care being taken that they were warm, after which they were placed with non-operated controls in stock cages and fed like the controls. Proper preoperative preparation and diligent postoperative care, with forced feeding, have been found by all workers to be of primal importance in working with suprarenalectomized animals.

Autopsies were performed on all rats and a careful search was made for accessory suprarenal tissue. Macroscopic accessory masses in the rat are not common;

1 Used by Professor Reid Hunt.
we have found them in about 3 per cent of our operated animals, and in rats that have not been subjected to suprarenaloection, they are found even more infre-
quently. When present these visible accessory masses are usually located in the vicinity of the kidneys. The thymus glands were removed from the rats as soon after death as possible and were dissected clean of the surrounding fat, connective tissue, and lymph nodes, and weighed.

The survival of suprarenaloectioned rats is explained generally on the assumption that these animals possess numerous accessory cortical rests, microscopic in size (17), located chiefly in close relation to the gonads. However, while survival may depend largely upon the presence of these microscopical accessories, we cannot but feel that additional factors come into play. A large proportion of rats and rabbits possess accessory tissue, but the rabbit is far less able to survive double suprarenaloection than the rat, and this dif-
ference cannot be explained solely by the amount of accessory tissue present. The suprarenal glands probably stand in different quantitative relationship to the vital bodily processes in different species.

We should like to emphasize here that our experimental and control rats were kept under identical laboratory and nutritional conditions, at all times, except that the operated rats received some special care during the immediate postoperative period. The standardiza-
tion of factors is extremely important in any research involving the thymus, for the gland is subject to tremendous, although to a con-
siderable degree controllable, variation.

EXPERIMENTAL DATA.

We have summarized our experimental data in several tables. Table I shows the results with 66 doubly suprarenaloectioned rats divided into two groups; namely, those dying after removal of both glands, and those which were sacrificed. Seventeen animals that died within 2 days following suprarenaloection are tabulated. The weights of their thymuses averaged 0.155 gm., while the weights of the thymuses of seventeen animals of the same ages as given by Donaldson would average 0.202 gm. Our average is, therefore, 23 per cent below Donaldson's for animals of the same age. We also sacrificed twenty-one non-operated animals in good condition and compared their thymuses with the Wistar standards for the
same age, and found that our controls averaged in weight 17 per cent below their figures. The fact that the weights of our normal thymuses are about 17 per cent below the Wistar standards is easily explained on the basis that our animals, while kept under good physical and nutritional conditions, were kept under different conditions from the Wistar rats.

We have included the thymuses of animals dying within 2 days after suprarenalectomy with those of our normal, sacrificed controls; we have done this because 2 days is insufficient time for detectable hyperplastic or significant involutionary changes to manifest themselves.

Five rats dying within 3 to 4 days after operation had thymuses 27 per cent below the Wistar figures, while nine dying within 5 to 6 days showed thymuses 39 per cent below these figures. These low figures are due, we believe, to additional factors, arising from the circumstances that suprarenalectomized rats, succumbing gradually in the course of some days, do not take food, and consequently thymus involution due to inanition and intoxication manifests itself. It is significant that our findings are in accord with the already well established fact that the moribund state is associated with diminution in the size of the thymus, and that our figures strongly controvert the findings of several previous observers who report large thymuses in animals which died for the most part within 6 days after bilateral suprarenalectomy.

In our series, one animal, dying of progressive insufficiency 9 days after operation, had a thymus 18 per cent above the Wistar figures. We cannot explain this except on the basis of physiological variation; its thymus may have been very large at operation. Four other rats, dying 12, 14, 25, and 29 days after suprarenalectomy from progressive suprarenal insufficiency and cachexia, presented thymuses 18, 23, 49, and 43 per cent below the Wistar figures. All four lost considerable weight after operation, and the last two at death had lost respectively 38 and 37 per cent of the weight normal to rats of their age.

We pass now to a consideration of the thirty animals which were killed by the injection of standard typhoid vaccine in the course of some immunological experiments, or were sacrificed from 9 to 207
days after suprarenalectomy (Table I). All animals included in this series which were killed with standard typhoid vaccine died within 6 hours after its administration and the thymus weights could not have been appreciably affected during this time interval. One is immediately impressed by the remarkable difference shown when their thymus weights are compared with Donaldson's standards for the same ages in normal rats. All thirty rats had thymuses above the Wistar standards. Two of these thirty were killed by vaccine 9 days after suprarenalectomy; they were in good condition at the time of injection and probably would have survived indefinitely. Their thymuses averaged 11 per cent above the Wistar standards.

<table>
<thead>
<tr>
<th>Cause of death</th>
<th>No. of rats</th>
<th>Averaged thymus weights, suprarenalectomized rats, at autopsy</th>
<th>Averaged thymus weights, normal rats, same age (Donaldson)</th>
<th>Percentage differences between thymuses of suprarenalectomized and normal (Donaldson) rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Died</td>
<td></td>
<td>gm.</td>
<td>gm.</td>
<td>per cent</td>
</tr>
<tr>
<td>1-2</td>
<td>17</td>
<td>0.155</td>
<td>0.202</td>
<td>-23</td>
</tr>
<tr>
<td>3-4</td>
<td>5</td>
<td>0.177</td>
<td>0.244</td>
<td>-27</td>
</tr>
<tr>
<td>5-6</td>
<td>9</td>
<td>0.123</td>
<td>0.203</td>
<td>-39</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>0.220</td>
<td>0.186</td>
<td>+18</td>
</tr>
<tr>
<td>12</td>
<td>1</td>
<td>0.141</td>
<td>0.172</td>
<td>-18</td>
</tr>
<tr>
<td>14</td>
<td>1</td>
<td>0.169</td>
<td>0.220</td>
<td>-23</td>
</tr>
<tr>
<td>25</td>
<td>1</td>
<td>0.103</td>
<td>0.203</td>
<td>-49</td>
</tr>
<tr>
<td>29</td>
<td>1</td>
<td>0.113</td>
<td>0.199</td>
<td>-43</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>0.200</td>
<td>0.180</td>
<td>+11</td>
</tr>
<tr>
<td>12</td>
<td>1</td>
<td>0.235</td>
<td>0.175</td>
<td>+34</td>
</tr>
<tr>
<td>18</td>
<td>2</td>
<td>0.297</td>
<td>0.162</td>
<td>+83</td>
</tr>
<tr>
<td>22</td>
<td>2</td>
<td>0.334</td>
<td>0.148</td>
<td>+125</td>
</tr>
<tr>
<td>25</td>
<td>1</td>
<td>0.235</td>
<td>0.170</td>
<td>+38</td>
</tr>
<tr>
<td>29</td>
<td>1</td>
<td>0.375</td>
<td>0.288</td>
<td>+80</td>
</tr>
<tr>
<td>30-35</td>
<td>5</td>
<td>0.326</td>
<td>0.150</td>
<td>+117</td>
</tr>
<tr>
<td>41</td>
<td>3</td>
<td>0.232</td>
<td>0.141</td>
<td>+65</td>
</tr>
<tr>
<td>52</td>
<td>4</td>
<td>0.209</td>
<td>0.155</td>
<td>+34</td>
</tr>
<tr>
<td>65-75</td>
<td>5</td>
<td>0.200</td>
<td>0.142</td>
<td>+40</td>
</tr>
<tr>
<td>170</td>
<td>1</td>
<td>0.160</td>
<td>0.085</td>
<td>+88</td>
</tr>
<tr>
<td>207</td>
<td>3</td>
<td>0.110</td>
<td>0.086</td>
<td>+16</td>
</tr>
</tbody>
</table>

TABLE I.
Another was killed by vaccine at 12 and still another 18 days after suprarenalectomy, and their thymuses were 34 and 83 per cent respectively above the Wistar figures. Two others died shortly after blood had been taken from the tail, as result apparently of struggling; their thymuses averaged 125 per cent above the Wistar standards. One rat was killed by vaccine 25 days after suprarenalectomy, while a second died 29 days after suprarenalectomy from exhaustion incident to fighting with another animal. Their thymuses were 38 and 30 per cent respectively above the normal weight. Five rats, sacrificed 30 to 35 days after operation, had thymuses which averaged 117 per cent above the normal. 41 days after operation 3 rats had thymuses averaging +65 per cent; at 52 days, 4 had thymuses averaging +34 per cent; at 65 to 75 days, 5 had thymuses averaging +40 per cent; at 170 days, 1 had a thymus which was +88 per cent; and at 207 days, 3 had thymuses averaging +16 per cent.

When our figures are corrected on the basis of a normal thymus weight about 20 per cent below Donaldson’s standards, the changes become even more significant, the actual increase in size of the thymuses of these thirty suprarenalectomized rats being about one-fifth more than the apparent increase.

Table II presents some individual data, summarized from the protocols. It has to do with twenty-seven of the thirty rats that were sacrificed or killed by vaccine. Analysis of this table yields the following information.

(1) Age.—One animal was operated on during the 2nd month of life, four during the 5th month, and twenty-two during the 6th and 7th months. Thus twenty-six of the twenty-seven were operated upon after thymus involution normally would have already begun, and of these, twenty-two when involution was probably already well established.

(2) Gain or Loss in Weight Following Operation.—Twenty-one of the twenty-seven were killed or sacrificed within 60 days after operation. Of these, eleven lost from 1 to 14 per cent of their original weight at operation, one did not change in weight, while nine gained from 1 to 22 per cent. The other six rats, sacrificed after 60 days, gained in weight. The significant feature is that 52 per cent of the
animals surviving double suprarenalectomy in good condition up to 60 days lost weight in spite of the fact that they were eating well, frequently much more than their controls, and had not attained their maximum weights at the time of operation. It has been pointed out by the early observers that animals surviving double suprarenalectomy for a week or more frequently lose weight rapidly and this loss of weight is due largely to the rapid disappearance of fat. This

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Sex</th>
<th>Age at operation</th>
<th>Length of time after operation</th>
<th>Weight at operation</th>
<th>Weight at death</th>
<th>Percentage gain or loss in weight since operation</th>
<th>Percentage differences between thymuses of suprarenalectomized and normal (Donaldson) rats of same ages.</th>
<th>Percentage differences between thymuses of suprarenalectomized and normal (Donaldson) rats.</th>
</tr>
</thead>
<tbody>
<tr>
<td>RW 12-2 M</td>
<td>162</td>
<td>9 days</td>
<td>262 gm.</td>
<td>230 gm.</td>
<td>−12 per cent</td>
<td>−1 per cent</td>
<td>+5 per cent</td>
<td></td>
</tr>
<tr>
<td>RW 12-1 &quot;</td>
<td>184</td>
<td>11 days</td>
<td>213 gm.</td>
<td>200 gm.</td>
<td>−6 per cent</td>
<td>−18 per cent</td>
<td>+18 per cent</td>
<td></td>
</tr>
<tr>
<td>RW 25-2 F</td>
<td>175</td>
<td>12 days</td>
<td>149 gm.</td>
<td>140 gm.</td>
<td>−6 per cent</td>
<td>−31 per cent</td>
<td>+34 per cent</td>
<td></td>
</tr>
<tr>
<td>RW 31-3 &quot;</td>
<td>184</td>
<td>18 days</td>
<td>212 gm.</td>
<td>202 gm.</td>
<td>−4 per cent</td>
<td>−3 per cent</td>
<td>+135 per cent</td>
<td></td>
</tr>
<tr>
<td>RW 31-4 &quot;</td>
<td>184</td>
<td>18 days</td>
<td>165 gm.</td>
<td>165 gm.</td>
<td>0 per cent</td>
<td>−21 per cent</td>
<td>+33 per cent</td>
<td></td>
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<tr>
<td>RW 16-3 &quot;</td>
<td>196</td>
<td>22 days</td>
<td>200 gm.</td>
<td>185 gm.</td>
<td>−7 per cent</td>
<td>−13 per cent</td>
<td>+111 per cent</td>
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<td>RW 16-2 &quot;</td>
<td>196</td>
<td>22 days</td>
<td>180 gm.</td>
<td>155 gm.</td>
<td>−14 per cent</td>
<td>−27 per cent</td>
<td>+140 per cent</td>
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<tr>
<td>RW 30-2 &quot;</td>
<td>168</td>
<td>25 days</td>
<td>176 gm.</td>
<td>170 gm.</td>
<td>−3 per cent</td>
<td>−17 per cent</td>
<td>+38 per cent</td>
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<tr>
<td>RW 3-2 M</td>
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<td>29 days</td>
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<td>+10 per cent</td>
<td>+13 per cent</td>
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<td>RW 33-1 F</td>
<td>178</td>
<td>31 days</td>
<td>200 gm.</td>
<td>245 gm.</td>
<td>+22 per cent</td>
<td>+16 per cent</td>
<td>+130 per cent</td>
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<td>178</td>
<td>31 days</td>
<td>210 gm.</td>
<td>235 gm.</td>
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<td>+11 per cent</td>
<td>+121 per cent</td>
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<td>150 gm.</td>
<td>165 gm.</td>
<td>+10 per cent</td>
<td>+13 per cent</td>
<td>+30 per cent</td>
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</tr>
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<td>200 gm.</td>
<td>245 gm.</td>
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<td>+16 per cent</td>
<td>+130 per cent</td>
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<td>RW 33-5 &quot;</td>
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<td>150 gm.</td>
<td>165 gm.</td>
<td>+10 per cent</td>
<td>+13 per cent</td>
<td>+30 per cent</td>
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<td>RW 33-6 &quot;</td>
<td>178</td>
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<td>200 gm.</td>
<td>245 gm.</td>
<td>+22 per cent</td>
<td>+16 per cent</td>
<td>+130 per cent</td>
<td></td>
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<td>RW 33-7 &quot;</td>
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<td>150 gm.</td>
<td>165 gm.</td>
<td>+10 per cent</td>
<td>+13 per cent</td>
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<td>RW 33-8 &quot;</td>
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<td>200 gm.</td>
<td>245 gm.</td>
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<td>+16 per cent</td>
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<td>RW 33-9 &quot;</td>
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<td>150 gm.</td>
<td>165 gm.</td>
<td>+10 per cent</td>
<td>+13 per cent</td>
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<td>200 gm.</td>
<td>245 gm.</td>
<td>+22 per cent</td>
<td>+16 per cent</td>
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<td>RW 33-11 &quot;</td>
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<td>150 gm.</td>
<td>165 gm.</td>
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<td>+13 per cent</td>
<td>+30 per cent</td>
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<td>200 gm.</td>
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<td>+16 per cent</td>
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<td>+13 per cent</td>
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<td>+16 per cent</td>
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<td>+16 per cent</td>
<td>+130 per cent</td>
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<td>+13 per cent</td>
<td>+30 per cent</td>
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<td>RW 33-20 &quot;</td>
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<td>200 gm.</td>
<td>245 gm.</td>
<td>+22 per cent</td>
<td>+16 per cent</td>
<td>+130 per cent</td>
<td></td>
</tr>
</tbody>
</table>
fact lends clinical support in the case of the rat to the findings of
Marine and Baumann (14, 18) and Scott (19), that removal or cripp-
ing the suprarenal glands in rabbits and cats frequently causes
a disturbance in metabolism characterized by a prolonged rise in
heat production. In this connection it is important to point out
that while cachectic and marasmic states ordinarily result in invol-
ution of the thymus, this organ enlarges after suprarenalectomy in
spite of the loss in weight, even when the animal is much below the
average weight for its age. We know of no clinical conditions except
Addison’s and Graves’ diseases, and occasional instances of status
lymphaticus, in which the thymus may enlarge in spite of a con-
siderable loss in weight.

(3) Curve of Thymus Enlargement.—The last column of Table II
would seem to indicate that thymic enlargement following supra-
renal ablation describes a curve. As has already been noted (Table I)
rats dying in from 5 to 6 days after bilateral suprarenal ablation
have thymuses much below the standard weight, while those sur-
viving operation 5 to 6 days in good condition, we believe possess
thymuses of normal size or even slightly enlarged. About this time
the stimulus for the thymic enlargement exerts a detectable effect,
the organ reaching its maximum size between the 3rd and 5th weeks
after suprarenal ablation. After the height of the curve is attained,
there is a gradual decline in the size of the gland, but for months
after operation it still does not approach that of the normal age
control. These changes may be interpreted on the basis that an
active secondary hyperplasia in the involuted thymus occurs soon
after bilateral suprarenal ablation, and that when the gland has
regenerated to that size which existing physiological and pathological
conditions permit, and then as compensation begins to influence it,
normal involution, though manifesting itself, is markedly delayed.

Table III shows the results in ten suprarenalectomized rats which
lived from 18 to 67 days after operation. Each rat is compared
with a control of the same, or approximately the same age. The
data bring out clearly the differences in the thymuses.
### Table III.

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Sex</th>
<th>Age at death (days)</th>
<th>Weight at death (gm.)</th>
<th>Cause of death</th>
<th>Length of time after suprarenalecotomy (days)</th>
<th>Weight of thymus (gm.)</th>
<th>Percentage differences between thymuses of suprarenalecotomized and control rats (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RW 31-3</td>
<td>F</td>
<td>202</td>
<td>202</td>
<td>Vaccine.</td>
<td>18</td>
<td>0.380</td>
<td>+162</td>
</tr>
<tr>
<td>RW 20-2</td>
<td>&quot;</td>
<td>199</td>
<td>155</td>
<td>Acute postoperative.</td>
<td>2</td>
<td>0.145</td>
<td></td>
</tr>
<tr>
<td>RW 16-3</td>
<td>&quot;</td>
<td>218</td>
<td>185</td>
<td>Died after a struggle.</td>
<td>22</td>
<td>0.313</td>
<td>+71</td>
</tr>
<tr>
<td>RW 39-2</td>
<td>M</td>
<td>209</td>
<td>285</td>
<td>Sacrificed.</td>
<td>Control.</td>
<td>0.174</td>
<td></td>
</tr>
<tr>
<td>RW 16-2</td>
<td>F</td>
<td>218</td>
<td>155</td>
<td>Died after a struggle.</td>
<td>22</td>
<td>0.355</td>
<td>+386</td>
</tr>
<tr>
<td>RW 37-1</td>
<td>M</td>
<td>211</td>
<td>210</td>
<td>Sacrificed.</td>
<td>Control.</td>
<td>0.073</td>
<td></td>
</tr>
<tr>
<td>RW 33-1</td>
<td>F</td>
<td>209</td>
<td>245</td>
<td>&quot;</td>
<td>31</td>
<td>0.359</td>
<td>+174</td>
</tr>
<tr>
<td>RW 32-1</td>
<td>M</td>
<td>209</td>
<td>245</td>
<td>&quot;</td>
<td>Control.</td>
<td>0.131</td>
<td></td>
</tr>
<tr>
<td>RW 33-4</td>
<td>F</td>
<td>209</td>
<td>235</td>
<td>&quot;</td>
<td>31</td>
<td>0.345</td>
<td>+130</td>
</tr>
<tr>
<td>RW 36-4</td>
<td>M</td>
<td>210</td>
<td>285</td>
<td>&quot;</td>
<td>Control.</td>
<td>0.150</td>
<td></td>
</tr>
<tr>
<td>RW 33-3</td>
<td>F</td>
<td>212</td>
<td>205</td>
<td>&quot;</td>
<td>34</td>
<td>0.415</td>
<td>-315</td>
</tr>
<tr>
<td>RW 32-2</td>
<td>M</td>
<td>212</td>
<td>210</td>
<td>&quot;</td>
<td>Control.</td>
<td>0.100</td>
<td></td>
</tr>
<tr>
<td>RW 30-4</td>
<td>F</td>
<td>209</td>
<td>175</td>
<td>&quot;</td>
<td>41</td>
<td>0.255</td>
<td>+88</td>
</tr>
<tr>
<td>RW 34-2</td>
<td>&quot;</td>
<td>209</td>
<td>185</td>
<td>&quot;</td>
<td>Control.</td>
<td>0.135</td>
<td></td>
</tr>
<tr>
<td>RW 29-3</td>
<td>M</td>
<td>209</td>
<td>242</td>
<td>&quot;</td>
<td>51</td>
<td>0.248</td>
<td>+72</td>
</tr>
<tr>
<td>RW 32-3</td>
<td>&quot;</td>
<td>209</td>
<td>250</td>
<td>&quot;</td>
<td>Control.</td>
<td>0.144</td>
<td></td>
</tr>
<tr>
<td>RW 16-1</td>
<td>F</td>
<td>261</td>
<td>295</td>
<td>&quot;</td>
<td>65</td>
<td>0.200</td>
<td>+78</td>
</tr>
<tr>
<td>RW 31-1</td>
<td>&quot;</td>
<td>264</td>
<td>202</td>
<td>Acute postoperative.</td>
<td>2</td>
<td>0.112</td>
<td></td>
</tr>
<tr>
<td>RW 17-1</td>
<td>M</td>
<td>210</td>
<td>225</td>
<td>Sacrificed.</td>
<td>Control.</td>
<td>0.225</td>
<td>+58</td>
</tr>
<tr>
<td>RW 17-4</td>
<td>&quot;</td>
<td>210</td>
<td>245</td>
<td>&quot;</td>
<td></td>
<td>0.142</td>
<td></td>
</tr>
</tbody>
</table>

**Unilateral Suprarenalectomy.**

We studied five unilaterally suprarenalectomized rats and did not observe in any of these the definite enlargement of the thymus that occurs after bilateral ablation, probably because little if any suprarenal insufficiency had been induced.
Sex Differences.

Of the twenty-five animals of Table II, which were killed or sacrificed 12 days after operation, seven were males. Their thymuses examined 29, 51, 52, 52, 52, 72, and 73 days after bilateral suprarenal ablation were 30, 59, 9, 50, 19, 28, and 12 per cent above the Wistar standards. On the whole, these thymuses are not as large as those of females of corresponding ages. We have not, however, sufficient data to establish this fact conclusively.

Pregnancy.

A previous pregnancy does not prevent the thymus from enlarging after suprarenalectomy. Seven of the eighteen females which were sacrificed had gone through a pregnancy before operation. Two were suprarenalectomized 2 weeks after casting litters and were killed by vaccine 18 days after operation. Their thymuses were 135 and 33 per cent above the Wistar standards. Two went through pregnancy 4 months before ablation of the glands and were autopsied 22 days after operation, their thymuses being 111 and 140 per cent above the Wistar standards. Three others were pregnant at various times before operation and were sacrificed 41, 65, and 170 days after suprarenalectomy. They also had large thymuses.

Chronic Lung Infection; Incomplete Removal of Suprarenals.

Animals presenting chronic lung infections were excluded from our series, since as is well known, pathological thymic involutions are rapidly induced by these infections. Nevertheless, we have seen large thymuses in a few bilaterally suprarenalectomized rats with chronic pulmonary infections. It would seem from this that the stimulus for hypertrophy and hyperplasia of the thymus following suprarenal ablation is so marked that involutionary changes may at times be counteracted.

The suprarenals are capable of undergoing rapid regeneration and when both glands are incompletely removed, the residual fragments may within a month attain a size even greater than that of the original glands. Rats examined a few months after incomplete suprarenalec-
show no enlargement of the thymus. If in such instances there had been thymus hyperplasia soon after operation, regression must have rapidly taken place.

**Histology.**

The tissues were studied histologically following Orth's fixation, paraffin embedding, and staining with hematoxylin and eosin.

Striking differences are apparent even in the gross between the regenerated thymuses of mature rats 30 or 40 days after suprarenallectomy and those of their non-operated age controls.

The glands of the former are large, white, and fleshy, while those of the latter are small, yellowish in color, and show fine connective tissue septa penetrating the organ. On histological examination the differences become even more apparent; the hyperplastic glands show large and closely apposed lobules, which are separated only by thin fibrous tissue septa. The bulging lobules are differentiated into distinct cortical and medullary zones, the cortical zones being thick and packed with closely aggregated small thymic cells, which occasionally show mitotic figures. The medulla is prominent, contains a few small thymic cells, a moderate number of reticular cells, and well formed Hassall's corpuscles. A number of our sections also show well formed, round, or irregularly alveolated, small and large duct spaces, lined by single or double layers of epithelium. Histologically, the large regenerated thymuses of mature suprarenalectomized rats resemble closely the growing thymuses of normal young animals, 50 to 70 days of age. One of the unmistakable evidences, however, that these large thymuses have regenerated, is the thickened arteries that are present in an otherwise plump, active gland.

Since our series includes thymuses examined from 9 to 170 days after suprarenalectomy, we have been able to trace the various steps in the secondary hyperplasia which follows suprarenal extirpation, and in the delayed involution that ensues after the regeneration of the thymus has become maximal.

The thymuses of the mature non-operated rats show involutionary processes which are manifested by a diminution in the amount of parenchymal substance, the appearance of a relatively abundant stroma, broadening of the septa, and the development of variable amounts of adipose tissue about the larger blood vessels. The vessels themselves show thickening of their coats. The differentiation between cortex and medulla is still present but both zones are reduced in size. In the more advanced involutions the lobules appear irregular in shape and reduced in size, the connective tissue about the blood vessels is more prominent and hyalinized, and the demarcation between cortex and medulla becomes obscure.

The thymuses of rats dying 5 to 6 days after operation show extensive disintegration of the small thymic cells, and blurring of the markings between cortex and medulla. These changes are dependent undoubtedly upon the toxemia and
Text-Fig. 1. Showing thymuses of twelve rats 208 to 212 days of age, six of which were suprarenalectomized 31 to 52 days before being sacrificed. One-tenth less than actual size.
acute inanition which generally are associated with the moribund state, and account for the marked diminution in weight of these thymuses below the Wistar standards.

Text-fig. 1 and Figs. 1 to 3 illustrate some of the changes described. The text-figure shows twelve thymuses of rats 208 to 212 days of age, six of which had been suprarenalectomized 31 to 52 days before being sacrificed, and their thymuses are compared with those of non-operated controls. Fig. 1 is a photomicrograph of the thymus of Rat RW 33-3, showing the marked regeneration and secondary hyperplasia. Fig. 2 shows the thymus of an age control (No. RW 37-1), with well established involution. Fig. 3 is a photomicrograph of the thymus of a rat 51 days of age, showing the striking resemblance between a normal growing and a secondarily regenerated thymus.

SUMMARY AND DISCUSSION.

Experimental evidence has been presented showing that the thymus gland of the rat enlarges rapidly in those animals surviving double suprarenalectomy. The enlargement has been observed to follow bilateral ablation with such constancy that it may be said to occur in all rats which survive double suprarenalectomy in good condition for from 3 to 5 weeks. The thymus enlarges even though the animal loses some weight after operation. Our data, though inconclusive, would seem to indicate a sex difference, the secondary hyperplasia of the thymus being more marked in the female than in the male. This difference, we believe, may shed some light on the mechanism of thymus enlargement following double suprarenalectomy. In the rat there is a definite sex difference in the weight of the suprarensals (20), the glands being much larger in the female than in the male. This weight difference becomes greater as the rat grows, and it appears at an early period of life; indeed, it is obvious at about 35 days of age, in spite of the fact that sexual maturity is seldom attained before 60 to 90 days. This difference in the suprarensals is independent of pregnancy. It is highly probable that the suprarenal glands stand in closer relation to the sex organs of the females than in the male, and that removal of these glands disrupts
The sex interrelationships between gonads, suprarenals, and thymus, this disturbance being more definitely expressed in the female than in the male.

The great majority of the rats were operated on when they were between 6 and 7 months of age; that is, after thymus involution would normally have set in. Thymuses examined when they had reached their largest size resembled both grossly and microscopically the growing thymuses of much younger animals, the only evidence of previous involutionary change being the presence of thickening of the vessels. We assume, therefore, from indirect, but statistical data, that regeneration of the involuted thymus must have taken place. The regeneration following bilateral ablation describes a curve which reaches its height between the 3rd and 5th weeks after operation. After the height of enlargement is attained, there is a gradual decline in the size of the gland, but involutionary changes may be retarded for months. Pregnancy prior to suprarenalectomy does not prevent thymus regeneration following this operation. Thymic enlargement does not ordinarily take place following a unilateral ablation. Severe chronic infections may sometimes bring about such a pronounced pathological involution of the thymus that the hyperplasia may be prevented.

There can as yet be only speculation as to the mechanism involved in the regeneration of the thymus which follows suprarenalectomy. This secondary thymic hyperplasia may be one manifestation of the generalized lymphoid hyperplasia that follows sublethal but sufficient suprarenal injury, and which is characterized by the appearance of prominent lymphoid foci in the various organs, particularly in the thyroid, a generalized hyperplasia of the lymph nodes and bone marrow, and the appearance of a lymphocytosis in the blood. The evidence at present available would suggest that both the thymic and general lymphoid hyperplasia may be effected through the gonads and parasex tissues and that the thymus especially regenerates because of a disruption of the interrelations between the interrenal gland, the gonads, and the thymus.

The close association between the gonads and the suprarenals (21) is well known. The latter undergo enlargement in pregnancy, ovulation, and castration, and the ovaries hypertrophy after sup-
rarenalectomy. Tumors of the suprarenals are associated frequently with sex perversion and precocity both in the male and female. The interrelation between the gonads and thymus is classical, castration before puberty delaying thymus involution for a long time.

A number of diseases present thymus enlargement among the major pathological findings at autopsy. The most outstanding of these are Addison's disease, status lymphaticus, and Graves' disease. It is now generally recognized that the suprarenal glands play an important rôle in the etiology both of Addison's disease and status lymphaticus. Whether the interrenal or the chromophil system is primarily involved or whether disturbances in function of both systems are at the basis of these diseases is still undetermined. The newer evidence, including our own work on lymphoid regeneration following suprarenalectomy supports the view that the lymphoid hyperplasia occurring in both Addison's disease and status lymphaticus is dependent upon insufficiency of the interrenal system (suprarenal cortex). While anatomical studies have not disclosed any constant lesions in the suprarenals in Graves' disease, evidence is being accumulated which would indicate that functionally these glands also play an important if not a primary rôle in the production of the clinical syndrome of Graves' disease (14, 18, 19). We are of the belief that the persistence of the thymus which occurs in status lymphaticus, and the regeneration which occurs in Addison's and Graves' diseases, are brought about by the same disturbances in glandular interrelations which bring about regeneration of the thymus in the experimental animal after suprarenalectomy.

CONCLUSIONS.

1. Secondary hyperplasia of the thymus resulting in enlargement of the organ follows, with great constancy, double suprarenalectomy in adult rats.

2. The height of the curve of this enlargement is reached in from 3 to 5 weeks after bilateral suprarenal ablation.

3. The enlargement seems to take place more readily in the female than in the male, is not prevented by a previous pregnancy, and occurs even when the animal has lost weight after operation.
4. The enlargement of the thymus following double suprarenalectomy is believed to result from a disturbance of the interrelations between the gonads, the thymus, and the interrenal gland (suprarenal cortex).

5. A possible relationship between the enlargement of the thymus occurring in Addison's disease, Graves' disease, status thymico-lymphaticus, and that following double suprarenalectomy is suggested.

I wish to express my sincere appreciation of the suggestive and stimulating criticism of Dr. Marine, which has made this work possible.

BIBLIOGRAPHY.

3. Averbeck, H., Die Addison'sche Krankheit, Erlangen, 1869.


EXPLANATION OF PLATE 13.

**Fig. 1.** Photomicrograph of the thymus of Rat RW 33-3, 212 days of age, showing marked regeneration and secondary hyperplasia. Hematoxylin and eosin. × 60.

**Fig. 2.** Photomicrograph of the thymus of Rat RW 37-1, non-operated control 211 days of age, showing well established involution. Hematoxylin and eosin. × 60.

**Fig. 3.** Photomicrograph of an actively growing thymus of a rat 51 days of age. Hematoxylin and eosin. × 60.
FIG. 1.

FIG. 2.

FIG. 3.

(Jaffe: Influence of suprarenal gland on thymus. 1.)