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Relation of Reticulo-Endothelial System to Refractoriness Developed in Response to Gonadotropic Hormone.

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We have presented evidence[1] to show that the so-called reticulo-endothelial system is intimately concerned with the development of refractoriness in rats to gonadotropic substance. We demonstrated that, whereas the ovaries of normal immature female rats, in response to daily doses of 10 R.U. of pregnancy urine extract, reach a maximum size after 10-15 days of treatment and then regress, the ovaries of splenectomized littermate animals continue to grow, attaining weights approximately 2 to 3½ times that of the treated controls, 20-30 days after beginning of injection. This increase in weight was shown to be due almost entirely to an increase in the size and numbers of corpora lutea. The ovaries of the splenectomized animals begin to regress after 30 days despite continued treatment. The interpretation given for these results was that with the extirpation of the spleen a large portion of the reticulo-endothelial system concerned with the production of the inhibitory substance for the luteinizing principle present in the pregnancy urine extract has been removed; this would therefore result in larger ovaries but that soon the function of producing the inhibitory substance would be taken over by other reticulo-endothelial elements in the liver, lymph and haemolymph nodes, etc., which are known and shown in our results to become hypertrophied and activated following removal of the spleen.

In the experiments described above and those to be presented below, only animals free of infection, especially *Bartonella muris*, a latent disease in rats, were employed. This is absolutely necessary since infection is a factor affecting reticulo-endothelial activity.

In order to further test our hypothesis that the activity of the reticulo-endothelial system is related to the loss of sensitivity following chronic treatment with heterozoic endocrine extracts, we have made a comparison of the abilities of plasma taken from treated splenectomized and control rats to neutralize the effects of

[1] Gordon, A. S., Kleinberg, W., and Charipper, H. A., *Science*, (in press).

injections of hormone in test animals. Three splenectomized and 3 control immature female hooded rats were injected with 10 R.U. Follutein daily for 20 days. At the end of this time they were subjected to light ether anesthesia and the blood, drawn by cardiac puncture, collected in oxalate. It was then centrifuged and the plasma pipetted off. Fourteen immature female hooded rats were used for testing the plasma. Seven of these were given injections of 0.1 cc. plasma of the treated normal rats along with 5 R.U. Follutein daily for 10 days, whereas the other 7 received daily injections of 0.1 cc. of plasma from the injected splenectomized rats plus 5 R.U. Follutein for the same period of time. The extract was injected on one side and the plasma on the other side of each animal to avoid mixing. At the end of 10 days the animals were sacrificed. The weights of the ovaries of animals injected daily for 10 days with 5 R.U. Follutein plus 0.1 cc. serum from 20-day Follutein treated normal rats were as follows: 44 mg., 48 mg., 43 mg., 78 mg.,* 47 mg., 39 mg., 43 mg.; average weight, 49 mg. The weights of the ovaries of animals injected daily for 10 days with 5 R.U. Follutein plus 0.1 cc. serum from 20-day Follutein-treated splenectomized rats were as follows: 82 mg., 60 mg., 72 mg., 59 mg., 87 mg., 83 mg., 91 mg.; average weight, 76 mg.

Although the number of test animals is relatively small, the results do seem to indicate that the plasma of 20-day injected control rats has inhibited the injected hormone preparation to a greater extent than the plasma of similarly treated 20-day splenectomized animals. This would account for the smaller size of the ovaries in the former as compared to the latter group of animals.

Further experiments were then conducted to determine whether the property producing the inhibitory substances to injected heterozoic hormone extracts is peculiar to the spleen or is possessed as well by the reticulo-endothelial elements in other organs. A group of 6 immature hooded female rats were splenectomized and then injected for 20 days with 10 R.U. Follutein. Beginning with the 20th day, and every second day thereafter, they were given intraperitoneal injections of trypan blue in physiological saline along with continued daily doses of 10 R.U. Follutein. The purpose of this was to see whether it would be possible to "block" with the injected dye, at least to some extent, the reticulo-endothelial compensation which develops and becomes obvious in the treated animals 20-30 days after removal

* This animal was the only one in the entire series which showed, at autopsy, a very large congested spleen, a sign of *Bartonella muris* infection. In the majority of such animals large ovaries result in response to treatment, due most likely to "blockage" of the reticulo-endothelial system.[1]

of the spleen. The animals were then sacrificed at intervals following this treatment, and the weights of the ovaries compared with those of Follutein-treated splenectomized animals not injected with the dye. The results are given in Table I.

TABLE I.

Immature splenectomized rats injected daily with 10 R.U. Follutein plus 1 cc. 0.5% trypan blue beginning with 20th day and every second day thereafter.			Immature splenectomized rats injected daily with 10 R.U. Follutein.		
No. Days After Splenectomy	No. of Animals	Wt. of Ovaries	No. Days After Splenectomy	No. of Animals	Wt. of Ovaries
		mg.			mg.
28-30	2	210-245	27-30	6	160-265
34-35	3	295-377	34-37	4	114-158
46	1	593	45-50	4	62-91

It is seen from this table that the trypan blue treatment has not only prevented the regression in size of ovaries which occurs rather rapidly in Follutein-treated splenectomized animals after 30 days, but in addition has resulted in a further considerable increase in size for at least 25 days beyond this period.† All the ovaries of the dye-injected animals show an increase in the numbers of large corpora lutea. This is similar to the response of ovaries in Follutein-treated animals whose reticulo-endothelial elements are blocked by the Bartonella organism.[1] The most obvious interpretation of these results is that the dye has produced a "blockage", in the splenectomized animals, of the remaining compensating reticulo-endothelial elements which normally take over the function of the spleen in inhibiting the action of the injected hormone. The treated splenectomized animals, given repeated injections of dye, are most likely producing much smaller quantities of the inhibitory principle for the hormone than the Follutein-treated splenectomized animals not injected with the dye.

Summary. 1. Immature splenectomized rats injected with pregnancy urine extract for 20 days produce a smaller quantity of inhibitory substance than normal injected controls. 2. "Blockage" experiments with trypan blue indicate that the production of antagonistic principle is not peculiar to the spleen but is possessed by the reticulo-endothelial system as a whole.

† The mortalities become high after repeated injections of the dye because of an increased susceptibility to infection.