Platelet Counts in the Rat After Hypophysectomy, Gonadectomy or Thyroidectomy.* (24168)

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It has been proposed that in mammals the number of circulating platelets, as well as erythrocytes and leukocytes, are under hormonal regulation(1-10). Bilateral removal of adrenal glands has been reported to cause an increase in platelet count(6-8); this increase has, however, been interpreted as due to surgical trauma(3). In human subjects administration of adrenal cortical extracts resulted in decreased platelet numbers(11), though no such effect was recorded following administration of adrenocorticotrophic hormone to either human beings or rats(7). Thus the role of adrenal hormones in control of circulating platelets has not been established. Reports are likewise conflicting on effects of gonadal steroids. Castration of rats resulted in decreased numbers of platelets, and administration of gonadal extracts in increased numbers(4). However, administration of large amounts of estrogens to either dogs or monkeys resulted in hypoplastic bone marrow and in a decrease in platelets(12). In human subjects injection of estrin and progesterone was reported to have no effect on the numbers of platelets(11), though more recently a decrease in platelets has been described after injection of estrogens, and an increase after injection of progesterone(10). Thyroxine has been reported to increase the number of platelets in human subjects(11); thyrotropic hormone also increased the number of blood platelets, but the increase appeared later and persisted longer(11). A significant decrease in platelet numbers has been described following removal of the pituitary(3).

In this study the role of pituitary, thyroid, and gonads has been reexamined in rats deprived of these organs. At the same time clot retraction was determined, in an effort to correlate numbers of platelets with this process.

Methods. Platelet counts were made weekly on male rats (Long-Evans strain) from the age of 5 weeks to 5 months. Ablation of endocrine organs was performed at 7 weeks of age.† *Hypophysectomy* was performed through the parapharyngeal approach and completeness of hypophysectomy was determined at autopsy by examination of sella turcica and by histological examination of target organs. No data from incompletely hypophysectomized rats is reported. *Castration* and *thyroidectomy* were performed surgically, the parathyroids being removed simultaneously with the thyroids. An intraperitoneal injection of 1 μc I^{131} was given 20 hours before autopsy. All tissue from the ventral cervical region was removed and radioactivity was determined in a scintillation counter (13). The radioactivity of a comparable piece of thigh muscle was subtracted from that of the thyroid region and the difference represented iodine uptake by any thyroid remnant. Counts of less than 0.01% of the injected radioactivity (or 0.1% of the normal uptake) were regarded as evidence of completeness of thyroidectomy. *Platelet* numbers were determined by the Rees-Ecker method (14). Blood was obtained by direct puncture of tail vein with a hypodermic needle, after coating the site with vaseline to prevent platelet injury. Blood was drawn from the freely

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† All thyroidectomized, gonadectomized, and normal rats were maintained on a modified McCollum Diet I consisting of ground whole wheat 67.5%, casein 15%, skim milk powder 7.5%, sodium chloride iodized 0.75%, calcium carbonate 1.5%, melted fat 6.75%, fish oil (Vit. A and D concentrate) 1%. KI was added to 1 μg of iodine/g of diet (450 mg KI/liter, 300 ml/270.5 lb of diet). Hypophysectomized rats were maintained on Diet XIV consisting of ground whole wheat 68.5%, casein 5%, alfalfa leaf meal 10%, fish oil 5%, NaCl 1.5%. KI was added to 1 μg of iodine/g of diet (450 mg KI/liter, 300 ml/270.5 lb of diet). In addition a wet mash of modified McCollum Diet I was fed daily. All animals received a supplement of lettuce 2-3 times a week, and were maintained at 74° ± 1°F.

TABLE I. Platelet Counts after Gonadectomy, Thyroidectomy or Hypophysectomy. Thousands/mm³; 6 rats/group.

	Weeks	Normal	Gonadectomized	Thyroidectomized	Hypophysectomized
Pre-oper.	1	538 ± 18.6*	558 ± 47.6	519 ± 32.7	509 ± 16.5
Post-oper.	1	597 ± 35.6	601 ± 23.6	477 ± 28.0	379 ± 2.0†
	2	499 ± 9.4	436 ±106.8	487 ± 32.4	383 ± 19.8†
	3	536 ± 42.2	381 ± 55.6	352 ± 74.0†	354 ± 52.8†
	4	490 ± 44.3	532 ± 45.6		381 ± 37.2†
	6	522 ± 26.0	425 ± 4.8		344 ± 51.2†
	12	552 ± 21.7	517 ± 13.9	375 ± 18.4†	371 ± 43.5†

* S.E. $= \sqrt{\frac{\Sigma d^2}{n(n-1)}}$. † $P < .01$.

bleeding wound into a Trenner automatic white blood cell diluting pipette; the blood was diluted immediately and shaken gently for 2 minutes. The first drops from the pipette were discarded; 15 minutes were allowed for platelets to settle in haemocytometer before counts were made. Duplicate determinations made from the same animal did not differ by more than 4%. *Clot retraction* was determined at the end of the experimental period by the method of Budtz-Olsen(15). Under ether anesthesia 5 cc of blood were drawn from the aorta into a paraffin coated syringe. A known quantity of blood was then allowed to run into a fluid of approximately the same specific gravity as of whole blood (mixture of liquid paraffin and trichloroethylene). The fluid containing the suspended sphere of blood was maintained at 37°C during clot retraction. The extruded serum of lower specific gravity rose to a higher level as the clot sank. When clot retraction was complete the expressed serum was removed by pipette and measured in a graduated cylinder. As a check the clot was lifted, washed in ether, and its volume determined by water displacement. Clot retraction was expressed as percent of serum extruded from whole blood.

Results. The effects of castration, thyroidectomy and hypophysectomy on numbers of blood platelets are shown in Table I. Only thyroidectomy and hypophysectomy resulted in significant decrease; platelet counts were reduced and eventually reached the same low level after 12 weeks. The decrease in platelet count occurred almost immediately after hypophysectomy, whereas after thyroidectomy it occurred only after the third week. No significant difference was observed in clot retraction in gonadectomized animals. but it was impaired in both thyroidectomized and hypophysectomized rats (Table II). The impairment was greater in thyroidectomized rats suggesting that the residual activity of thyroid after hypophysectomy was able to influence clot retraction. The change in clot retraction is consistent with the assumption that a reduction in platelets impairs normal retraction of the blood clot(5).

TABLE II. Clot Retraction after Gonadectomy, Thyroidectomy or Hypophysectomy.

Group	No. of rats	Clot retraction, % serum
Normal control	6	42 ± 3.0
Gonadectomized	6	44 ± .8
Thyroidectomized	6	22 ± 1.5
Hypophysectomized	3	33 ± 3.4

The number of circulating platelets is therefore controlled, at least in part, by pituitary and thyroid secretions. As the number of circulating platelets is decreased after thyroidectomy as well as after hypophysectomy it is probable that the reduction in platelets after hypophysectomy is secondary to thyroid deficiency, however it can not be ignored that alterations in pituitary function occur after thyroidectomy, and reduction in numbers of platelets may result from this alteration.

It should be noted that besides platelets the only formed elements in blood which decrease in numbers after hypophysectomy are erythrocytes.

Summary. Removal of pituitary gland, or of thyroid resulted in decrease in number of

circulating blood platelets. Decrease in clot retraction observed in these animals was consistent with reduced platelet counts. Gonadectomy had no effect on post-operative platelet counts or clot retraction.

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Influence of X Irradiation on Potassium Retentivity by *Escherichia coli.** (24169)

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Hevesy and Zerahn (quoted by Hevesy (1)) observed that the K^+ efflux from yeast increased after X irradiation. Bruce and Stannard(2,3) made a detailed study of the influence of radiation on K^+ efflux and influx in yeast. The present study is an extension of their work to bacteria. Although a number of different species were tested, this report will be concerned only with *Escherichia coli.* Strain B and its radiation-resistant mutant B/r are compared, and some other factors that influence K^+ retentivity by *E. coli* are discussed.

Methods. Escherichia coli was cultivated in broth of the following percentage composition: glucose, 1; $(NH_4)_2HPO_4$, 0.4; KH_2PO_4, 0.1; $MgSO_4 \cdot 7H_2O$, 0.07; and sodium citrate, 0.05. Glucose and ammonium phosphate were autoclaved separately as 20 and 8% solutions, respectively, then added to the autoclaved and cooled basal solution. A 12-hour, 500-ml culture was decanted into 5 liters of fresh broth contained within a 12-liter round-bottom flask in 37°C water bath. The resulting mixture was continuously aerated through a 4-mm bore glass tube at 2½ liters of air per minute. After 12-14 hours incubation, the cells were harvested in a Sharples centrifuge, then mixed with enough distilled water to form about 40 ml of thick suspension. This will be designated as stock suspension. Cells were washed in centrifuge once with distilled water, twice with 0.05 *M* sodium phosphate (pH 6.2) and, after the last centrifugation, were made up to 200 ml in this buffer. The suspension was divided in half and each 100-ml portion was placed

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