the anti-egg antibody. This result was observed upon incubation in 0.1 M acetate buffers (pH's 4.0 and 4.6). Incubation in 0.1 M phosphate buffers (pH's 6.6 and 7.3) showed the same result but required a longer time of hydrolysis. The pH of fractions incubated in acetate buffers was brought back to about 7.4 by addition of dilute NaOH prior to performance of the circumoval test. These results indicate that the anti-egg precipitin present in humans infected with *S. mansoni* is a gamma globulin with a sedimentation coefficient of 6.6 S; an approximate molecular weight calculated from sedimentation and diffusion data close to 200,000; and its physical chemical architecture and chemical integrity are needed to yield a positive circumoval reaction.

Summary. The gel filtration technique was used to fractionate samples of sera from 6 individuals infected with *S. mansoni*. The gel used, Sephadex G-200, allows the separation of serum into 3 fractions. The individuals studied showed a marked increase in the 7 S globulins. The specific antibody which was present in the serum of these individuals and which has been found to react with the *S. mansoni* ova yielding a positive circumoval precipitin test was found to be a normal size gamma globulin with a sedimentation coefficient of 6.6 S and a molecular weight close to 200,000. Partial enzymatic hydrolysis of this protein was found to alter its immunologic properties, rendering the circumoval test negative after incubation with reactive ova, indicating that the chemical integrity of this protein is needed to yield a positive circumoval reaction.

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Electrolyte and Water Exchanges in the Estrogen Treated Ovariectomized-Adrenalectomized Rat.* (29737)

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The reductions in fluid exchange and electrolyte excretion obtained in diethylstilbestrol (DES) treated ovariectomized rats has been attributed primarily to a decrease in food ingestion(1). However, a reduction in food intake cannot account for the depression of the urinary Na:K ratio observed, for pair-feeding, alone, did not affect the ratio. While this finding indicates a possible specific DES effect, direct or indirect adrenal cortical involvement was not excluded. Other investigators have found that electrolyte excretion in adrenalectomized rats is not altered by low or moderate doses of estrogens(2,3,4). Deming and Luetscher(2) obtained a sodium retaining effect only when the estradiol was administered in relatively large doses. In the experiments with adrenalectomized rats, the observation period was limited to 3 to 7 hours after estrogen administration. Others have obtained positive results in intact species(5,6,7,8,9,10) only after a more prolonged period of treatment(18-19 hours).

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TABLE I. Average Daily Food and Water Intakes and Urine Volumes for Days 6 and 7 in Ovariectomized-Adrenalectomized Rats.

	Food			Water			Urine		
	g*± SEM	p	n	ml*± SEM	p	n	ml*± SEM	p	n
Normal	6.4 ± .3	ns	22	10.8 ± .6	ns	24	3.7 ± .3	.02	24
Ovx + Adrx (control)	5.4 ± .4	—	16	11.5 ± .6	—	16	2.2 ± .5	—	16
Idem + 10 μg DES	1.0 ± .2	.01	18	4.7 ± .5	.01	18	1.7 ± .3	ns	18
" + 25 μg "	.8 ± .2	.01	16	4.3 ± .8	.01	16	1.2 ± .5	ns	16
" + 50 μg "	.6 ± .2	.01	17	2.4 ± .3	.01	17	.3 ± .1	.01	17
" + 100 μg "	.6 ± .2	.01	10	2.1 ± .5	.01	10	.1 ± .04	.01	10
" + pair-fed (to 100 μg group)	.7 ± .1	.01	16	5.4 ± .6	.01	16	.6 ± .2	.01	16

* g or ml per 100 g body wt. SEM = standard error of mean. n = No. of observations. Ovx + Adrx = ovariectomized-adrenalectomized. DES = diethylstilbestrol. ns = difference not significant.

Accordingly, the influence of DES on electrolyte and water exchanges in the adrenalectomized rat was reevaluated under conditions of measured food intake and a more prolonged period of treatment.

Methods. Sherman rats weighting 175-190 g were bilaterally ovariectomized, adrenalectomized, and immediately housed individually in metabolism cages. Daily measurements of body weight, food and water intakes, and of urinary volume and electrolytes were begun 24 hours later. All measurements were made at approximately the same time each morning for a 7-day period. Details of the collection procedures have been published(1). A dry pellet form of diet (Rockland Rat Diet—D free) was used; the electrolyte content was periodically determined and found to be relatively uniform (Na^+ 0.5, K^+ 0.17, Cl^- 0.47 mEq/g). Urinary sodium and potassium determinations were made by flame photometry, and urinary chloride was determined by the Cotlove chloridometer. On day 7, blood samples were obtained by cardiac puncture for duplicate micro-hematocrit and serum electrolyte analyses. A sample of the left gastrocnemius muscle was removed for tissue water determination.

Diethylstilbestrol was administered subcutaneously in 0.2 ml of sesame oil daily for 7 days in dosages of 10, 25, 50 or 100 μg. A group of ovariectomized-adrenalectomized rats was pair-fed to the rats receiving 100 μg DES to determine the influence of the estrogen-induced reduction in food intake, *per se*. Intact normal rats were simultaneously observed.

The results, except where indicated, are expressed as the average of the 6th and 7th days. This was done in order to minimize the daily variations, to illustrate the cumulative effect, and to reduce the volume of data to be reported. The overall (entire week) averages are in agreement with the combined 6th and 7th day results. The untreated ovariectomized-adrenalectomized group served as controls for statistical comparison.

Results. Ovariectomy-adrenalectomy caused an immediate and continued reduction in food intake which resulted in a loss of body weight. Treatment with all dosages of DES resulted in a marked further reduction in food ingestion, so that the body weights progressively fell to about 85% of their first day weight as did the pair-fed group.

The average relative water intake (per 100 g body weight) for the ovariectomized-adrenalectomized animals was not significantly different from that of normal females. DES administration at all dosages caused significant decreases ($p<.01$) in relative water intake, as did pair-feeding (Table I). The latter, however, did not have as great a reduction as was seen in the 100 μg DES treated group ($p<.01$).

As a consequence of the above changes in water and food ingestion, water/food (W:F) ratio was increased ($p<.01$) in all estrogen treated groups. The W:F ratio (4:2) for the 100 μg DES treated group was significantly

lower than in the pair-fed control (6.4) owing to the greater reduction in water intake. The normal group had a W:F ratio of 1.7. This was lower ($p<.01$) than that for the ovariectomized-adrenalectomized controls.

Ovariectomy-adrenalectomy caused a significant decrease ($p<.02$) in urine volume when compared to the normal rats (Table I). Although 10 and 25 μg DES dosages further decreased urine volume, only the 50 and 100 μg DES dosages caused significant reductions ($p<.01$). The reduction ($p<.01$) in urine volume caused by pair-feeding was significantly less than that induced by administration of 100 μg DES.

There were no statistically significant changes in urinary Na^+, Cl^- and K^+ excretion in the ovariectomized-adrenalectomized animals when compared to normal animals (Table II). DES administration at all dosages, resulted in significant reduction ($p<.01$) in urinary Na^+, Cl^- and K^+ and these reductions were proportional to the estrogen dosage. Pair-feeding also caused a significant reduction ($p<.01$) in urinary electrolyte excretions, but this reduction was significantly less than that obtained in the 100 μg DES treated group. When expressed as the per cent of the sodium and chloride intake which is excreted, the 50 and 100 μg DES dosages reduced the excretion to 31 and 17%, respectively, while pair-feeding did not alter the excretion from the normal value of 40%.

DES, at all dosage levels, resulted in a reduced ($p<.01$) urinary Na:K ratio, while the ratio for the animals pair-fed to the 100 μg DES treated group did not differ from the normal group value (2.0). The reduced urinary Na:K ratios in all estrogen treated animals were brought about by the greater reduction in Na^+ than K^+ excretion (Table II).

For all groups, the average values for tissue (gastrocnemius) total water content ranged from 76.4 to 77.0%. There was no significant difference among these groups. Ovariectomy-adrenalectomy, treatment with 10, 25, 50 or 100 μg DES and pair-feeding increased the hematocrit above normal (44); average values being 48, 51, 56, 53, 58 and 59 re-

TABLE II. Average Daily Urinary Electrolyte Excretions for Days 6 and 7 in Ovariectomized-Adrenalectomized Rats.

	Sodium			Chloride			Potassium			Na:K		
	mEq ± SEM	p	n	mEq ± SEM	p	n	mEq ± SEM	p	n	± SEM	p	n
Normal	1.26 ± .10	ns	20	1.45 ± .06	ns	23	.64 ± .04	ns	23	2.0 ± .2	ns	20
Ovx + Adrx (control)	1.19 ± .08	—	13	1.27 ± .08	—	13	.54 ± .04	—	13	2.2 ± .1	—	13
Idem + 10 μg DES	.32 ± .06	.01	18	.33 ± .06	.01	18	.23 ± .03	.01	18	1.4 ± .1	.01	18
" + 25 μg "	.16 ± .04	.01	16	.16 ± .04	.01	15	.12 ± .02	.01	16	1.2 ± .1	.01	15
" + 50 μg "	.13 ± .03	.01	17	.11 ± .03	.01	15	.12 ± .02	.01	15	1.1 ± .1	.01	15
" + 100 μg "	.05 ± .01	.01	10	.06 ± .02	.01	10	.05 ± .01	.01	10	1.0 ± .2	.01	10
" + pair-fed (to 100 μg group)	.25 ± .03	.01	16	.21 ± .03	.01	16	.14 ± .02	.01	15	1.9 ± .2	ns	15

mEq = milliequivalents per 100 g body wt. SEM = standard error of mean. n = No. of observations. Ovx + Adrx = ovariectomized-adrenalectomized. DES = diethylstilbestrol. ns = difference not significant.

spectively. Ovariectomy-adrenalectomy significantly reduced ($p<.02$) the serum Na^+ from a normal of 145 to 135 mEq/l, and neither estrogen administration nor pair-feeding altered this effect.

Discussion. Previous studies had shown that DES, at 50 and 100 μg dosage levels, decreased the urinary Na:K ratio in ovariectomized rats and that this effect was independent of the depressed food ingestion (1). In the present studies, DES at all dosages (10, 25, 50 and 100 μg) significantly reduced the urinary Na:K ratio in the absence of adrenal cortical tissue. Furthermore, this effect for the 100 μg dosage was independent of altered electrolyte intake as demonstrated with pair-feeding. These results are in agreement with those of Deming and Luetscher(2), who obtained sodium retention, but only with extremely large doses of estradiol in adrenalectomized rats. The failure to obtain an effect with lower doses of estradiol may be due to the short-term nature of their experiments; the measurements were conducted 5½ hours after the subcutaneous estrogen administration. This may also account for the negative results obtained by others(3,4) when the estrogen effect on electrolyte excretion in adrenalectomized rats was evaluated 3 or 7 hours after injection. The present findings are also in agreement with the results obtained in the dog(9,10). Thorn and Engel demonstrated that Amniotin administration to the adrenalectomized male dog partially prevented the initially increased excretion of Na^+ and Cl^- which normally follows loss of adrenal cortical function(9).

There is indirect evidence of an effect of estrogen on renal function independent of adrenal mediation. Estradiol administration to the female patient with Addison's disease resulted in a marked and prolonged retention of Na^+, and Cl^-(9,10). However, in such cases, the results are not unequivocal as the amount of remaining functional adrenal cortical tissue is not known. It has also been established that estrus prolongs survival of adrenalectomized animals(11,12).

While the present studies provide evidence that DES *per se* may effect a renal conservation of salt, it should not be reasoned that at these estrogen dosages such salt-saving could sustain the adrenalectomized rat. Indeed, at these dosages the toxicity of DES overshadows any beneficial effect. Of the animals receiving 100 μg, one-third survived the 7th day of observation, while only ⅔ of those receiving 10, 25, or 50 μg survived. On the other hand, all of the control ovariectomized-adrenalectomized and those pair-fed to the 100 μg group survived the 7-day experimental period. It cannot be ruled out that the lowered Na:K ratio may be due to the toxic effect of this compound.

The lowest dosage used (10 μg) is above the physiological estrogen secretion rate in the rat(13). This dosage induced a 14% loss in body weight. It remains to be determined whether or not a lower dosage of this synthetic estrogen or of a natural estrogen would influence urinary Na:K under these experimental conditions.

Diethylstilbestrol depressed water intake as well as food intake. This may account for the consistently lower body weight in the 100 μg DES treated animals than in their pair-fed controls despite a lesser water excretion in the former. This may also explain the significantly lower W:F ratio in the 100 μg DES treated rats compared with their pair-fed controls.

The lack of change in total water content of the gastrocnemius muscle samples does not exclude some change in the extra-intracellular distribution. The failure to find a change in muscle water content 7 days following adrenalectomy is in keeping with the data of others(14). The increase in hematocrit suggests a possible shift of fluid into the cells. The estrogen (100 μg DES) did not alter either the increased hematocrit or the lowered serum Na^+, indicating that the decreased electrolyte and water excretions were not sufficient to compensate for the reduced intake. Since the experiment was of only 7 days duration, there probably was insufficient time for more marked changes in water redistribution or compensation.

The mechanism by which the DES depressed sodium excretion is not known. Since DES did not alter glomerular filtration in the ovariectomized rat(1), and since the DES

induced reduction in electrolyte excretion in the dog was not related to changes in glomerular filtration or renal plasma flow(6), the estrogen effect is probably not mediated by changes in renal hemodynamics. The fact that both the estrogen treated and pair-fed animals had the same serum electrolyte levels, coupled with the fact that estrogen did not alter filtration rate in the ovariectomized animal, would imply that the filtered load might be the same. While an altered tubular reabsorption in the estrogen treated animal is suggested, the effect of estrogen on filtered load and reabsorption in the adrenalectomized rat remains to be determined.

Summary. Daily administration of 10, 25, 50 or 100 μg of diethylstilbestrol (DES) to overiectomized-adrenalectomized rats for 7 days significantly reduced the Na^+, Cl^- and K^+ excretions and the urinary Na:K ratio. Pair-feeding (to the 100 μg DES group) also reduced the electrolyte excretions, though significantly less than that of the 100 μg DES treated animals, but did not alter the urinary Na:K ratio which remained normal. Thus, at these dosages, DES can effect a diminished electrolyte excretion in the absence of adrenal cortical tissue and independently of electrolyte intake.

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Influence of Genotype on Muscle Regeneration in Mice. (29738)

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Skeletal muscle is one of the regenerative tissues in mammals(1-4). In the past few years, in particular, a body of evidence has emerged from several independent laboratories indicating that the primary events are essentially the same as those that operate in differentiation of muscle of the embryo(2-4). It seemed quite possible that the amount of regeneration in a muscle would show a relationship to pedigree. The specific objective of this work was to find out if genetically different strains of mice would exhibit different levels of regeneration.

Materials and methods. Experiments were performed with male Jackson Memorial Laboratory mice approximately 3 months of age, from one of the following strains: $B6AF_1$, SJL/J, C57BL/6J and A/J(5). Strains C57BL/6J and A/J are purebreds. Strain $B6AF_1$ is a hybrid from matings between C57BL/6J females and A/J males. Strain SJL/J is an independent line of Swiss Webster mouse. At time of experimentation animals weighed 23-25 g.

Mice were anesthetized lightly with methoxyflurane and 1 mm $\times$ 1 mm transections