(ACTH) administration can increase the concentration of red blood cells in the peripheral blood of mice within 3-4 hours after administration(8). Unfortunately, the role of the pituitary-adrenal axis in altering platelet counts is not clear. Though exogenous ACTH produced no changes in platelet counts of rats(9,10), exogenous corticosteroids(11,12) as well as adrenalectomy(10) have caused thrombocytosis.

A delayed thrombocytopenia (Table I) occurred 7 days after hyperthermia (day 8). The platelet counts of control animals which had remained relatively unchanged until days 7 and 8 had significantly increased on these days (Table I). The fact that these increases had not occurred in the experimental animals indicates the suppression of changes in platelet counts in the heat stressed animals. It would seem that the delayed thrombocytopenia was caused by the decreased production and/or release of new platelets into the general circulation, which takes about one week(13).

Conclusions. Exposure of male rats to hyperthermia (55°-57°C) for a 15 minute period caused an immediate thrombocytosis lasting for 1 hour after removal from heat stress, a delayed thrombocytosis starting 3 hours after removal from heat stress and lasting through day 2, and a thrombocytopenia appearing 7 days after heat stress. Speculation as to the different physiological mechanisms which might have caused the thrombocytosis at these 2 time periods points toward an immediate release of platelets from their followed by a hemoconcentration due to the pituitary-adrenal stress response. The delayed thrombocytopenia has been attributed to decreased production and/or release of new platelets.

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Some Metabolic Aspects of Calcium Movement Across the Isolated Avian Shell Gland.* (32455)

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The avian shell gland has as one of its primary functions the deposition of $CaCO_3$ to form the egg shell. Studies of the shell gland in the domestic fowl have shown that Ca distribution in this organ is related to egg position in the oviduct. The calcium level in the shell gland was lowest when an egg shell was forming and highest when the egg was in the magnum and infundibulum sections of the oviduct(1). In fractionation studies of the shell gland muscosa of ^{45}Ca-treated hens, the ^{45}Ca content of mitochondria, which was higher than that of other cell fractions on a nitrogen basis, decreased when a shell was being deposited(2). Oxygen uptake studies of the oviduct revealed that the uptake was highest in the shell gland and maximal in this

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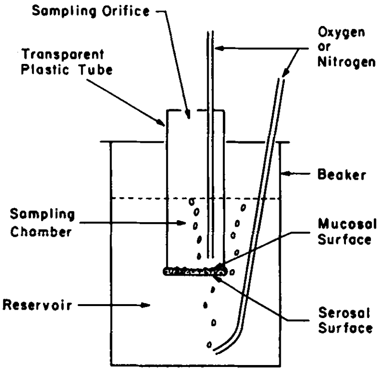
segment just prior to receiving an egg(3).

The present study was undertaken to clarify further the nature of the relationship between the metabolic activity and the movement of calcium through the shell gland tissue.

Methods and materials. Twenty-eight regularly laying single-comb White Leghorn hens, maintained on a standard laying ration and a regular artificial light shedule, were used. All had well-established laying records and, with the exception of 2, were in their first year of laying. The use of birds in their second year caused no change in the data. Hens were decapitated, the oviducts were removed, and the location of the egg was noted. The shell gland was excised, blotted on tissue paper, and cut along the longitudinal axis into two segments. These segments were rinsed in buffer solution identical to the incubation medium, but without glucose, at 39.5°C. Both pieces of tissue were stretched and tied over the open ends of identical transparent plastic tubes, 1.94 cm in diameter. The closed ends of the tubes contained openings for aeration and sampling. The preparations were placed in 100 ml beakers; 5 ml of incubation medium without tracer were placed into each tube to form the sampling compartment; 75 ml of medium with approximately 40 μC of ^{45}Ca in each beaker constituted an "infinite" reservoir. A diagram of the system is shown in Fig. 1. The medium bathing each membrane surface was stirred by a continuous stream of pure O_2. To avoid hydrostatic pressure gradients, the liquid surfaces of sampling compartment and reservoir were maintained at the same level by adjusting the position of the tubes. Incubation was conducted at 39.5 ± 0.5°C for a period of about 7 hours. "Infinite" reservoir values were established by sampling before and after incubation. In studies of the effects of various physical and chemical conditions on ^{45}Ca movement from serosa to mucosa, the mucosal surfaces of both membranes faced the interior of the tubes. Unidirectional Ca fluxes were determined from paired membrane preparations by mounting one of the segments in the usual manner to determine influx from serosa to mucosa and inverting the other to measure efflux from mucosa to serosa.

The incubation medium had the follow-



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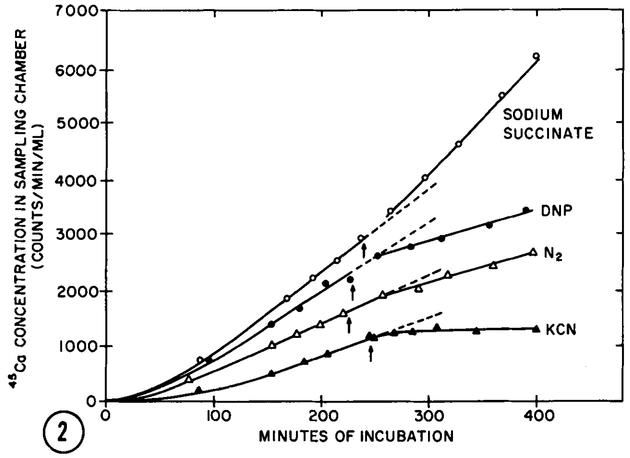

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FIG. 1. Diagram of apparatus used for determining the unidirectional ^{45}Ca fluxes of shell gland preparations *in vitro*. The entire apparatus is immersed in a water bath at 39.5°C. ^{45}Ca is added to the incubation medium in the reservoir. Substrates and inhibitors are added and samples are removed through the sampling orifice. As shown, the tissue is oriented for the study of ^{45}Ca flux from the serosa to the mucosa. To measure flux ratios, one of the pair of membranes cut from the shell gland is mounted with its mucosal surface inverted.

FIG. 2. Typical response curves of ^{45}Ca movement from serosa to mucosa are shown for sodium succinate (○), DNP (●), N_2 (△) and KCN (▲). Curves were selected to prevent graphic overlapping, and each represents data from a different experimental animal. Arrows indicate initiation of treatment. Preparations required more than 100 min to achieve the initial steady-state transfer rates and about 20-30 min to reach steady-state rates resulting from treatment.

ing composition: NaCl, 136 mM; KCl, 22.8 mM; $CaCl_2$, 1.80 mM; $MgSO_4$, 1.66 mM; Tris(hydroxymethyl)aminomethane, 23.8 mM, and glucose 16.7 mM, which was added to the medium before each experiment. The Ca concentration, approximately equal to the ionic plasma Ca concentration and about one-third of the total plasma Ca concentration of laying hens(4), was verified by titration with EDTA and calcein indicator(5). The pH was adjusted to 7.45 at 39.5°C, and the medium was made 11% hypertonic to body fluid to reduce tissue swelling(6). Substrates or metabolic inhibitors were added in 0.1 ml volumes to the incubation medium bathing the mucosal surface of the membrane.

In the study of the effects of various agents or changes in environment on transmural Ca movement, membranes were taken when the shell gland contained a partially calcified eggshell (soft or brittle shell) at the time of sacrifice. Each membrane was used as its own control by determining the rate of ^{45}Ca movement both before and after treatment. Aliquots of 0.1 ml were withdrawn periodically from the sampling chamber, dried on stainless steel planchets, and analyzed for ^{45}Ca in a Geiger-Mueller counter. The activity of each aliquot was corrected for volume changes by sampling and treatment and expressed as the concentration of ^{45}Ca in counts/min/ml present in the sampling chamber. Evaporation was negligible. The corrected data were plotted as a function of time, and the slopes of the straight line portions of the curves before and after treatment were determined by the method of least squares. Unidirectional fluxes of stable Ca (μmole/cm^2/hr) were derived from the slopes in the usual manner(7,8).

Results and discussion. The responses of the membranes to the various treatments *in vitro* are shown in Table I. The data demonstrate that, when a calcified eggshell was present in the shell gland at sacrifice, Ca influx is dependent on the availability of oxidizable substrate, such as sodium succinate, and is markedly decreased during inhibition of oxidative metabolism by potassium cyanide (KCN), 2,4-dinitrophenol (DNP), and N_2 atmosphere. The variations in the degree of response can be explained in part by unavoidable differences in the distention of the membranes. Some typical response curves are shown in Fig. 2. Each curve represents data from a different experimental animal. Individual variations among the animals and differences in the membrane preparations, such as the degree of stretching, may account for the spread in the magnitudes of the Ca influxes prior to treatment. To exhibit the effects of the various treatments on Ca movement more clearly, the examples in Fig. 2 were selected from the individual graphs to avoid overlapping of the Ca influx curves.

TABLE I. Effect of Various Chemical and Physical Conditions on ^{45}Ca Influx When an Egg with a Calcified Shell Was in the Shell Gland. The response values are the mean percentage changes in the influxes caused by treatment (+ SEM). Plus and minus signs denote an increase or decrease of movement. Numbers in parentheses represent No. of preparations.

Treatment	Concentration* (molarity)	Response (%)
Sodum succinate	1×10^{-2}	$+ 33 \pm 12$ (6)
KCN	5×10^{-2}	$- 88 \pm 11$ (5)
DNP	5×10^{-5}	$- 28 \pm 8$ (5)
Nitrogen atmosphere		$- 34 \pm 11$ (4)

* Final concentration present in medium bathing mucosal surface of membrane.

The average unidirectional fluxes and net transfer of Ca across the shell gland *in vitro*, calculated from the data of the paired membrane preparations, are presented in Table II. Also shown are the flux ratios and their relationship to the location of the egg in the oviduct at time of sacrifice. When membranes were taken for study when there was no egg in the shell gland at time of sacrifice, the net flux is zero and the flux ratio is 1.0. Specifically, the movement of Ca was nondirectional, whether the egg was located in the isthmus or magnum portions of the oviduct, or when no egg was present in the oviduct. When the shell gland contained a partially calcified eggshell, a net movement of Ca occurred from serosa to mucosa as shown by a flux ratio of 2.4. In 2 cases which are not entered in Table II, the shell glands contained small and soft-shelled eggs which appeared to be plumping at time of sacrifice. In these preparations, influx/efflux ratios of 1.0 and 1.1 were obtained as in the no-egg case. In the

TABLE II. Unidirectional Calcium Fluxes Across the Isolated Shell Gland and Their Relation To Egg Location in Oviduct at Time of Sacrifice (Mean ± SEM).

Egg position	No. of observations	Calcium flux (μmole/cm^{2}hr) Influx	Efflux	Net influx	Flux ratio (Ca influx/ Ca efflux)
Not in shell gland*	5	.032 ± .007	.031 ± .005	.001 ± .004	1.0 ± .1
In shell gland	6	.051 ± .004	.021 ± .001	.030 ± .004	2.4 ± .2

* In 3 cases the oviduct did not contain an egg; in one case an egg was in the magnum and in another it was in the isthmus.

absence of concentration gradients and isolation from $CaCO_3$ deposition *in vitro*, the net Ca movement seems to parallel the time course of shell deposition *in vivo*. In the live animal, the rate of deposition is initially low, followed by an increase to a maximal and constant value toward the end of the plumping period (4).

The results indicate that only when an egg was in the shell gland at time of sacrifice was a net flux of Ca observed *in vitro*. Furthermore, net movement of Ca occurred in the absence of an eggshell and isolation from the physiological variables present in the live animal. The data suggest the existence of a physiological control mechanism which, at the time the egg enters the shell gland or sometime thereafter, initiates net movement of Ca in the mucosa.

Summary. The experiments indicate that calcium movement across the avian shell gland *in vitro* is in part dependent on metabolic energy derived from oxidative metabolism, requires the generation of phosphate-bond energy, and is predetermined by the physiological state of the shell gland at time of sacrifice.

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Relationship Between Circulating Red Cell Volume and Endogenous Thyroxine in Sheep.* (32456)

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Relationships of the thyroidal system to the red cell-hemoglobin axis was recently discussed by Muldowney *et al*(1) who observed red cell mass to be reduced in hypothyroid human subjects. In newborn rats, Adam and Doljansky(2) noted the development of a progressive hyperchromic-microcytic anemia following thyroidectomy. Falkheden *et al*(3) reported red cell volume and total hemoglobin to be reduced after hypophysectomy in 14 human subjects affected by mammary carcinoma, acromegaly or diabetes. These data, however, suggested that thyroid deficiency alone did not explain the observed results. In a later report by Meineke and Crafts(4), an erythropoietic effect of thyroxine was demonstrated in rats that was indepen-

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