quence(12). It was suggested that the blockade of OIH release by the same gonadotrophic preparation (LH) so effective in forcing ovulation under differing conditions might be the outcome of increased blood estrogen levels, but we have no direct evidence that this is so. It would be of much interest to know estrogen levels not only before and after gonadotrophin administration, but also during the course of the specific activity consequent upon electrical stimulation in the hen.

Our results do not exclude the possibility that stimulation at other regions of the hen's brain, or with current of differing parameters, may induce OIH release in the hen as it does in some mammalian species. The single forced ovulation recorded in Table I may be of some import in this connection, since LH release at 4:00 p. m. of the terminal day of the cycle is not encountered normally. The differing response of the hen to stimulation may nevertheless betoken differences in organization and function of nervous or endocrine factors in birds and mammals. It is of interest in this connection that some barbiturates regularly blocking OIH release in the rat may induce OIH release prematurely in the hen(13).

Summary. Bilateral stimulation with bipolar stainless steel electrodes was applied for 10 minutes in a selected region of the median eminence of the hen, 14 hours before expected normal ovulation of the C_1 follicle. In 28 of 52 hens, ovulation was delayed by 3 to 10 hours or more. The delays in ovulation were shown to arise out of delays in or blockade of the release of ovulation-inducing hormone (OIH). The effects of stimulation on delay of OIH release may extend over a period of 12 to 14 hours. Stimulation with platinum electrodes or placement of steel or platinum electrodes without passage of current failed to delay OIH release. The prolonged effects of stimulation with stainless steel electrodes are consistent with the conclusion of Everett and Radford that electrolytically deposited iron may act irritatively long after application of the stimulatory current.

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Discrimination in Intestinal Absorption of Strontium and Calcium.* (26919)

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The preliminary results here reported represent an effort to study the effect of calcium on absorption of trace levels of radiostrontium from the intestinal tract of rats using a method which focuses attention on the absorption process. To eliminate the influence of excretory and bone deposition processes, many recent studies of intestinal absorption have been conducted *in vitro,* using the everted intestinal-sac technic described by

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Wilson and Wiseman(1). Schachter and coworkers, in a series of papers, have used this technic to study factors which affect the transport of calcium from the small intestine of the rat(2,3,4). An active transport system seems to be involved in the absorption of a part of the calcium, and this system appears not to be involved in strontium absorption. Wasserman has more specifically compared the absorption of strontium and calcium, using the intestinal-sac technic, and has shown that strontium absorption, unlike calcium absorption, is insensitive to prior history of dietary calcium levels(5).

Curran and Solomon have devised a technic of perfusing a segment of the intestine, *in vivo*,(6) which they have used in studying absorption of calcium and strontium in rats. This technic is a more physiological approach to the problem than the intestinal-sac technic and provides a measure of intestinal absorption which is for most purposes not significantly affected by the subsequent fate of the absorbed material in the animal. Using this perfusion technic, Dumont, Curran and Solomon studied the absorption of both calcium and strontium as a function of their concentration in the lumen of the intestine(7). Their observations on the absorption of strontium were compatible with a passive diffusion process. The absorption of calcium showed evidence, at higher intraluminal calcium levels, of an active transport process. These studies involved perfusion of the entire ileum plus a portion of the jejunum. Wasserman *et al.* have recently reported the results of a similar study, with calcium only which, however, involved perfusion of a segment of the duodenum(8). The active transport of calcium was much more evident in these duodenal preparations, as would have been predicted from intestinal-sac results(2,9).

The present studies were not designed to elucidate absorption mechanisms but to measure the discrimination between strontium and calcium in the process of absorption from the intestine, and to determine the effect on this discrimination of varying the level of calcium intake. Data were also obtained on the movement of intravenously injected strontium and calcium into the intestine.

Methods. Female albino rats of the Sprague-Dawley strain were fasted for 24 hours in preparation for the perfusion studies. They had previously been maintained on Purina Lab Chow (calcium content, 1.3%). The entire small intestine was perfused under pentobarbital anesthesia. The inflow cannula, inserted through the mouth, was tied in place in the upper duodenum, through an abdominal incision. The outflow cannula was inserted into the small intestine and tied in place at the ileo-cecal junction. The intestine was disturbed as little as possible and left in the animal during perfusion. The incision was covered with saline-moistened gauze and temperature of the animal maintained with heat lamps. In one series of experiments the large intestine was cannulated in a similar manner and perfused from cecum to anus.

The perfusion solution was maintained at body temperature, and circulated by means of a peristaltic action pump. The perfusion rate employed was approximately 1.0 ml/min and the perfusion pressure did not exceed about 7 cm of water. The total perfusion period in all experiments was 100 minutes. Preliminary experiments had shown that rate of absorption of Sr^{85} or Ca^{45} was independent of rate of perfusion in the range from 0.5 to 1.5 ml/min, and remained reasonably constant for perfusion periods of up to 100 minutes. The perfusion solutions were 5 mM in KCl, 20 mM in glucose, and 0, 1, 5 or 25 mM in $CaCl_2$. Strontium-85 was added as a solution of the nitrate; Ca^{45}, as a solution of the chloride. The quantity of non-radioactive strontium and calcium associated with the radioisotopes in no case exceeded a final concentration of 1 μg/ml in the perfusion solution. Total osmolarity of all solutions was adjusted to 300 osmM with NaCl. pH was adjusted to approximately 7 with NaOH.

In the first series of experiments, Sr^{85} and Ca^{45} tracers were added to the perfusion solutions at levels of approximately 0.1 μc/ml. The animals were first perfused for 25 minutes with a solution containing no radioisotopes; the same solution, but containing Sr^{85} and Ca^{45}, was continued for the next 50 minutes, followed for a final 25-minute flush with

the radioisotope-free solution. Animals were sacrificed at conclusion of the perfusion and radioisotope content determined in the total animal (exclusive of small intestine) and in the effluent perfusion solution. In the second series of experiments, Sr^{85} and Ca^{45} in amounts of approximately 2 μc, (approximately 7 μg non-radioactive calcium, less than 1 μg non-radioactive strontium) were injected into the tail vein 50 minutes after the start of perfusion. The perfusion was continued for an additional 50 minutes following which the animals were sacrificed and radioisotope content determined in the total animal (exclusive of tail), in the segment of tail adjacent to the injection site, and in the effluent perfusion solution. If more than 2% of the injected radioisotope was found at injection site at the end of experiment, the data were discarded.

Sr^{85} was determined by gamma-scintillation counting in a sodium-iodide well-type counter. Following determination of Sr^{85}, the samples were ashed and aliquots counted for the Ca^{45} betas in a thin-window proportional counter. The Sr^{89} impurity in the Sr^{85} was shown to contribute insignificantly to the Ca^{45} count.

Results and discussion. Data on absorption of Sr^{85} and Ca^{45} from the perfused small intestine as determined from analyses on the total animal are shown in Table I. When expressed as per cent of total radioisotope administered, absorption of both Sr^{85} and Ca^{45} is inhibited by addition of calcium to the perfusion solution. In terms of total calcium absorbed, there is, of course, an increase as the calcium level in the perfusate is increased. The absolute rate of calcium absorption (Table I), is calculated from the radioisotope data on the assumption that the Ca^{45}/total calcium ratio in the solution perfusing the intestine remained constant during transit. This is not strictly true, since unlabeled endogenous calcium is excreted into the intestine throughout the perfusion. Rates of total calcium absorption are in reasonable agreement with those obtained in earlier studies, being lower by a factor of 2 at the 1 mM calcium level than the duodenal rate measured by Wasserman *et al.*(8); and somewhat higher at the 1 and 5 mM calcium level than the rates measured by Dumont *et al.* in the ileum and jejunum(7). The rate at 25 mM calcium is considerably lower than the average value of 0.9 μM/hr cm measured by Dumont *et al.* At this high calcium level the movement of calcium into the intestine has been shown to be appreciable(7), and the assumption of constant specific activity used in estimation of our total calcium absorption values is apt to introduce the greatest error.

TABLE I. Absorption of Sr^{85} and Ca^{45} from the Perfused Small Intestine of the Rat (Average Values from 6 Rats ± Stand. Dev.).

Conc. of Ca in perfusate (mM/l)	% of perfused radioisotope absorbed			Total Ca absorbed* (μM/hr cm)
	Sr^{85}	Ca^{45}	Sr^{85}/Ca^{45}	
0	4.3 ±.6	14.9 ± 2.3	.29 ±.04	—
1	3.2 ±.6	7.3 ± 1.6	.45 ±.04	.04
5	2.6 ±.3	4.8 ± .4	.54 ±.06	.14
25	1.4 ±.1	2.2 ± .2	.63 ±.04	.33

* Estimated on the assumption of constant Ca^{45}/Ca^{40} ratio in solution passing through intestine.

Of more significance to the present investigation are the ratios of Sr^{85}/Ca^{45} absorbed. These numbers accurately reflect the relative behavior of exogenous calcium and strontium and indicate not only a discrimination against strontium in the absorption process, but a discrimination which changes as a function of calcium intake. These results are in accord with observations on continuous feeding of radiostrontium to rats, where the ultimate deposition of strontium and calcium in bone showed a similar pattern of variable discrimination as a function of dietary calcium level (10,11). The inference may be drawn that the pattern of strontium and calcium deposition is determined largely by the discrimination which occurs in the absorption process. Discrimination may occur in other physiological processes. It would appear, in fact, that greater discrimination occurs in the renal excretory processes(11). However, the discrimination which occurs in absorption from the intestine is totally effective, in that all ingested strontium is subjected to this process; whereas, that which occurs in the kidney affects only that fraction of the ingested strontium which ultimately passes through this organ.

The results listed in Table I might be in-

TABLE II. Excretion of Intravenously Injected Sr^{85} and Ca^{45} into the Perfused Intestine of the Rat (Average Values from 3 to 5 Rats ± Stand. Dev.).

Segment perfused	Perfusate [Ca] (mM)	% of inj. radioisotope excreted		
		Sr^{85}	Ca^{45}	Sr^{85}/Ca^{45}
Small intestine	0	3.1 ± .1	2.6 ± .3	1.2 ±.2
	1	4.2 ±1.2	4.1 ±1.1	1.0 ±.1
	5	2.6 ± .3	2.5 ± .3	1.0 ±.2
	25	1.9 ± .2	1.9 ± .2	1.0 ±.2
Large intestine	1	.5 ± .3	.4 ± .3	1.3 ±.4

terpreted in a very different manner if a substantial portion of the absorbed radioisotopes were re-excreted into the intestine. Thus, one might assume that the intestine was completely permeable to both strontium and calcium, and that the observed discrimination was due to selective binding of calcium on bone. That such is not the case was shown in a second experiment in which Sr^{85} and Ca^{45} were introduced into the blood and their appearance measured in the perfusate. The results (Table II) indicate that no very substantial fraction of Sr^{85} or Ca^{45} reaching the bloodstream is excreted into the intestine. The apparent decrease in movement of Sr^{85} and Ca^{45} into the intestine with increasing levels of calcium in the perfusion solution was unexpected, in view of the reported increase in movement of calcium into the intestine under these conditions(7). The explanation would seem to lie in the fact that the Ca^{45} in this experiment cannot be considered as a tracer for blood calcium, and while the ultimate effect of an increased calcium load in the blood may be to return a larger portion of calcium to the intestine, the immediate effect would be to accelerate the deposition of this calcium on bone, where the Ca^{45} label would be diluted with the freely exchangeable calcium of bone.

Of particular interest in Table II are the Sr^{85}/Ca^{45} ratios in the perfusate, which are in no case significantly different from unity. There would appear to be no discrimination between strontium and calcium in this process of excretion into the intestine. To establish this point with certainty it would have been necessary to measure blood levels of Sr^{85} and Ca^{45} during the course of perfusion. This was not done. Some further evidence for a lack of discrimination in this process was provided, however, by the fact that the perfusate when collected and analyzed in successive 10 ml portions, showed no variation in the ratio of Sr^{85}/Ca^{45}, with time of collection, although the total quantities of radioisotopes fell sharply after collection of the first 10 to 30 ml.

The very small amount of Sr^{85} and Ca^{45} excreted into the large intestine (Table II) indicates that no appreciable error was introduced by excluding this section of the intestine from the remainder of the study.

A word of caution is in order concerning the interpretation of these experiments. Although the technic of *in situ* perfusion of the intestine is clearly more physiological than the study *in vitro* of an intestinal preparation, there remain important non-physiological features. The composition of the perfusing solution is rather markedly different from the normal content of the intestine, and the uniform rate of passage through all segments of the tract is unlike the situation which normally pertains. A more detailed study of the problem would involve perfusion of separate segments of the intestine, weighting the results from each segment in the light of normal rates of passage through that segment (12). Despite these limitations the data indicate, at least semi-quantitatively, the importance of the absorption process in the discrimination between strontium and calcium and the variability in this discrimination which occurs as a function of calcium intake level.

Summary. Absorption of Sr^{85} and Ca^{45} was studied in the rat by the technic of *in vivo* intestinal perfusion. With no calcium added to the perfusion solution the percentage of Sr^{85} absorbed was about 0.3 that of Ca^{45}. With increasing levels of calcium in the perfusion solution, the percentage of Sr^{85} and Ca^{45} absorbed decreased; however, the ratio of percentage absorption of Sr^{85} to Ca^{45} increased, reaching a value of about 0.6 in 25 mM calcium solution. There was no evidence of discrimination in the reverse movement of Sr^{85} and Ca^{45} from blood to intestine. The discrimination which occurs in the absorption

process would appear to be a dominant factor in determining over-all strontium calcium relationships.

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Coenzyme Q Concentration in Proliferative Lesions of Liver.* (26920)

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Histochemical studies of the succinic dehydrogenase system using tetrazolium salt reduction have shown a decrease of succinate-tetrazolium reductase activity in a number of benign and malignant proliferative lesions (1,2,3). Recent work employing the tetrazolium salt, 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT), has indicated that a member of the coenzyme Q group of quinones serves as an intermediate electron transport agent in the succinate-INT reductase reaction, and that in many tissues this system is not saturated with respect to quinone(4,5,6). Accordingly, a decrease in succinate-INT reductase activity might be due to either a decrease in activity of the primary dehydrogenase, a lack of availability of quinone, or some combination of the two effects. An effort was made to ascertain which of these alternatives exists in regenerating liver and the Novikoff hepatoma by addition of coenzyme Q_{10} to the reaction mixture. It was found that with frozen sections of the Novikoff hepatoma addition of coenzyme Q_{10} to the succinate-INT system resulted in a considerably greater enhancement of reductase activity than occurs in normal liver(5). This suggested that the coenzyme Q content of this neoplasm might be relatively low. In contrast, in regenerating liver addition of coenzyme Q_{10} to the reaction mixture caused an enhancement of activity of approximately the same order of magnitude as that of normal liver. These findings suggested that the coenzyme Q content of regenerating liver was not significantly reduced, but that some other factor was causing the decrease in activity observed. Since the enhancement of activity of the succinate-INT reductase activity by addition of coenzyme Q_{10} to the reaction mixture can be considered at best as supplying only indirect evidence of the relative amount of coenzyme Q available for the reductase reaction, direct quantitative studies of the coenzyme Q content of these tissues were undertaken. The results clearly demonstrate that the Novikoff hepatoma and the more slowly growing Morris hepatoma #5123 have a considerably lower concentration of coenzyme Q than normal liver, while in regenerating liver the coenzyme Q concen-

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