sone, cortisone, amphenone, hyypophysectomy, reserpine, hexestrol, O', P'-DDD, restraint) some of which altered the concentration in liver(15).

Although the hepatic enzyme systems responsible for glucuronide formation have been studied extensively in mammals and identified in the pigeon(16), no data are available concerning their presence in adrenal tissue. β-glucuronidase, responsible for hydrolysis of glucuronides, has been reported to have a wide distribution in various organs (including the adrenal) in both mammals and birds. The presence of the enzymes controlling both synthesis and hydrolysis of steroid glucuronides in adrenal tissue, together with the demonstration that a large percentage of glucuronide-bound steroid occurs in the avian adrenal during normal and experimental conditions (6,15), strongly suggests that the steroid glucuronides play a role in adrenocortical metabolism in the bird. The nature of this role remains to be investigated.

Summary. Total homogenates and microsomal fractions of avian adrenal glands were incubated with cortisone, cortisol, hydrocortisone or hydrocortisol in the presence of excess uridine diphospho-glucuronic acid. The resulting data indicate the presence of an enzyme system in the microsomes capable of conjugating all the above compounds except cortisone with glucuronide with liberation of uridine diphosphate. The presence of β-glucuronidase was also demonstrated in avian adrenal tissue. These results, together with the previous demonstration that significant amounts of glucuronide-bound steroids are present in the avian adrenal gland under normal and various experimental conditions, imply that steroid glucuronide conjugation and hydrolysis play some role in the normal physiology of this gland.

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Effect of Warfarin (Coumadin) Sodium Administration During Lactation on Blood Coagulation of Nursling Rats.* (26000)

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The anticoagulant warfarin (Coumadin) sodium has come into wide use for treatment and prevention of various thromboembolic disorders, including those in postpartum patients(1,2). This latter application has raised the question of the drug's effect in lactation, *i.e.,* whether or not anticoagulant therapy for the mother would have an adverse effect on the nursling. This problem has now been investigated by determination of whole blood clotting time by the capillary method in mother rats fed diets containing warfarin

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TABLE I. Toxic and Therapeutic Warfarin Sodium Dosage Levels.

Warfarin sodium, % diet	Mothers		Nurslings	
	Survival	Clotting time, min.	No.	Clotting time, min.
.01	1/3	>390	3	>260
.005	3/5	>100	13	38 (10 -140)
.001	2/5	>100	22	6.4 (4 - 10)
.0005	2/2	50 (10 - 90)	9	3.1 (1 - 10)
.00025	3/3	5.7 (4.5- 7)	33	1.1 (1 - 1.5)
.000125	11/11	3.1 (3 - 3.5)	87	1.0 (.5- 1.5)
None	11/11	1.1 (.5 - 1.5)	75	1.0 (.5- 1.5)

sodium, and in their nurslings.

Materials and methods. The rats used were CF albinos (Carworth Farms). In the various experiments mothers were alternately put on stock diet or warfarin sodium (WS) diet on the same day that the litters were born. The stock diet was ground Purina Lab Chow checkers. WS diets were prepared by dissolving the anticoagulant in acetone, adding the solution to a small amount of ground stock diet, grinding well in a mortar, and finally mixing the concentrate thoroughly with the required amount of stock diet in a Hobart mixer. Food consumption was recorded. The experimental diets were fed for 7 or 14 days. The experiments could not be carried beyond 14 days because after that age the nursling rats were large enough to crawl near the solid diet. At the end of experimental periods mothers and nurslings were etherized, and blood samples were taken by exposing the heart, snipping the left ventricle with a scissors, and filling a capillary glass tube† (0.04 mm I.D., 90 mm long) with the freely flowing cardiac blood. Whole blood clotting time was determined by breaking the capillary tubing at intervals until a fibrin thread was observed. Intervals of 15 seconds were used in the principal experiments, and longer intervals in the exploratory work. All determinations were made at the controlled room temperature of 25 ± 1 C. Prothrombin determinations were made on some of the mother rats, blood sample being drawn from the heart by syringe. The Quick one-stage method(3) was used, but with a nichrome stirring loop to get the plasma clotting endpoint.

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Results. Determination of toxic and therapeutic dosages. In Table I are shown results of the 7-day range-finding experiments. At a WS level of 0.01% of the diet, only 1 out of 3 mothers survived, and the survivor had a tremendously prolonged clotting time of more than 390 minutes. Survival incidence in their nurslings was not meaningful, because of the poor condition and deaths of the mothers. However, the 3 surviving nurslings all showed greatly prolonged clotting times, greater than 260 minutes, and gross evidence of hemorrhage beneath the skin of the neck and at other sites. This demonstrated that at WS levels highly toxic or lethal for the mother, the anticoagulant action was transmitted through the milk. Gross hemorrhage was also seen in 2 of the 13 surviving nurslings at the next lower level of 0.005%, but was not seen below that dosage.

The anticoagulant effect in both mother and nurslings decreased with decreasing dosage. However, at all dosage levels there was much less anticoagulant effect on nurslings than on mothers. Thus, at 0.00025% WS all 33 nurslings had normal clotting times of 1.0-1.5 minutes, whereas the 3 mothers had an average clotting time of 5.7 minutes (range 4.5-7), or about 5 times normal. At the dosage level of 0.000125% clotting time of the mothers was 3.1 minutes (range 3-3.5), which was 3 times the average normal and double the 1.5 minute upper limit of normal clotting range. According to Connell and Mayer(4), the therapeutic range for clinical anticoagulation is about 1.5-2 times normal whole blood clotting time by their method. Therefore, the WS level of 0.000125% was considered to be at least equal to therapeutic anticoagulation of the mothers and was selected for further investigation and analysis.

TABLE II. Comparison of Clotting Times of Mothers and Nurslings at Therapeutic Warfarin Sodium Dosage Level.

Warfarin sodium, % diet	No. days	Mothers: No. animals	Mothers: Clotting time, min.	Nurslings: No. animals	Nurslings: Clotting time, min.
None	7	11	1.1 ± .1 *	75	1.02 ± .07*
.000125	7	11	3.1 ± .1	87	.97 ± .04
None	14	16	.95 ± .07	123	.97 ± .04
.000125	14	16	3.1 ± .1	120	.95 ± .04

* Mean ± S.E.

Comparisons at therapeutic dosage. At the therapeutic WS dosage level of 0.000125% clotting times of mothers and their nurslings were compared after 7 days and after 14 days of feeding. In Table II are given mean clotting times and standard errors. In both experiments clotting time of the WS mothers was about 3 times that of the stock diet control mothers. By the "t" test these differences were found to be highly significant statistically ($P = <0.001$ in each experiment). However, clotting times of the WS nurslings were normal, in fact, very nearly the same as those of control nurslings. Clotting time for the WS mothers was practically the same after 14 days as after 7 days, indicating that the 0.000125% dosage was essentially a stabilized maintenance level. Prothrombin activity was determined on a group of 10 WS mothers and averaged about 25% (range 15-32%) of the levels in mothers on normal stock diet, thus corresponding to a therapeutic or prophylactic level. At the dietary WS level of 0.000125%, average daily food consumption of the nursing mothers was about 25 g, equivalent to a WS intake of approximately 0.16 mg/kg of body weight. No significant pathology was found in WS mothers or nurslings, when autopsied after clotting time had been taken.

Growth and reproduction of warfarin sodium nurslings. Further studies were made on the nurslings to determine whether or not there would be delayed deleterious effects. After 14 days on the therapeutic level of 0.000125% WS, 4 mothers were transferred to stock diet and allowed to complete weaning of their litters. Likewise, 4 control mothers on the stock diet were allowed to wean their litters. The male and female WS nurslings showed normal weaning weights, growth, and reproduction (Table III). The second generation young in turn were weaned and grew normally. On autopsy at termination of experiment, the parents and the second generation young appeared normal. Thus, ingestion of the therapeutic anticoagulant level of WS by lactating mothers produced no delayed deleterious effects in offspring.

Discussion. A certain amount of confusion has existed regarding the effects on the young of anticoagulant administration to the mother during pregnancy and during lactation. In clinical and animal studies with the older anticoagulant, bishydroxycoumarin, it was indicated that the fetus or newborn was more susceptible to anticoagulant action than the mother(5). Special caution is recommended in using anticoagulants antepartum(6). When bishydroxycoumarin was administered to lactating rats, bleeding was caused in the nursing young; however, the dosage used was so high that it was lethal to the mothers(7). It was concluded from later experiments in dogs that it would probably be quite safe to administer bishydroxycoumarin to the mother during lactation because little of the anticoagulant gets

TABLE III. Growth and Reproduction of Nurslings from Mother Rats Fed Therapeutic Level of Warfarin Sodium.

Warfarin sodium in diet, %	At weaning (3 wk): No. litters	No. young	Avg wt of young, g	Avg wt gain in 9 wk, g: ♂	♀	Reproduction: No. mothers	No. litters	No. young	Avg weaning wt, g
None	4	46	31	238	153	12	12	84	37
.000125	4	37	34	274	161	11	11	97	37

into the milk(5). In subsequent clinical studies of nursing mothers, it was reported that therapeutic dosages of bishydroxycoumarin could be administered without danger of hemorrhage or effect on prothrombin time in the nursing infants(8,9). The present experiments on warfarin sodium in rats are consistent with the above results, and suggest that therapeutic dosages of warfarin sodium could probably be administered to nursing mothers without deleterious effect on the infants.

Summary. When a therapeutic dosage of the anticoagulant warfarin (Coumadin) sodium was fed to lactating rats, there was no effect on whole blood clotting time of the nurslings, or on their subsequent growth and reproduction. When toxic or lethal levels of warfarin sodium were fed to the mothers, clotting time was prolonged in the nurslings. This demonstrated that the anticoagulant effect could be transmitted through the milk when mothers received toxic dosages. However, at all dosages the anticoagulant effect was much less in nurslings than in mothers.

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Site of Formation of Thyroglobulin in Mouse Thyroid as Shown by Radioautography with Leucine-H^3.* (26001)

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Locating sites of protein synthesis in the thyroid gland has been attempted after injection of S^{35}-labelled methionine(1,2), but may now be done better with tritium (H^3)-labelled amino-acids, since the soft beta-rays of tritium allow a more precise radioautographic localization than those of S^{35}. When mice were injected with methionine-, glycine- or leucine-H^3, the location of the H^3 label in the thyroid was found to be the same with the 3 amino-acids but reactions were more intense with leucine-H^3 than with the other 2. This last result was not unexpected since leucine has been listed(3) as the most abundant amino-acid in thyroglobulin, the characteristic iodinated protein found in the thyroid colloid. The next step was to investigate quantitatively the thyroid gland of those mice which had been treated with leucine-H^3. This was done in the present investigation.

Methods. Ten adult male mice weighing 26-32 g received a single injection of 5 μc of DL-leucine-4, 5-H^3 (150 mc/mM) per gram body weight and were sacrificed in pairs at ½, 4 and 35 hours, 7 and 45 days later. Thyroid glands were fixed in Bouin, stained with hematoxylin-eosin and radioautographed(4).

In one section of each thyroid gland, the largest and smallest diameters of the colloid of every follicle were measured with a micrometer; and photographic grains were counted within a 10.4 x 10.4 μ area over the center of the colloid. Grains were then counted within 2 x 10.4 μ areas selected over 2 diametrically opposed portions of the epithelium of each follicle. Finally, grains were also counted in 10.4 x 10.4 μ areas located outside the thyroid

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