apparently not as great in development of Arthus reactions as in passive cutaneous anaphylaxis, and also the ease of depleting an animal of factors essential for inflammation might well vary among different species.

2). The finding reported here would also suggest that an inhibitor, such as reported by Hayashi(10,11), does not play a substantial role in cessation of vascular inflammation, at least under the experimental conditions in our study. It should be noted that a fresh, acute reaction developed in vessels about which mononuclear cells, without doubt left over from the previous reaction, were abundant. This occurred even when small amounts (27 μg NBSA) of antigen were used. It is very possible that the inhibitor reported by Hayashi, which he indicates is related to the mononuclear cells, is overwhelmed by the inflammatory reaction that occurs following fresh antigen-antibody combination in vessel walls and with the subsequent influx of polymorphs.

Thrombosis of vessels in the Arthus reactions unquestionably plays an important role in development of the macroscopic lesion, and resolution of these thromboses is most certainly a factor in termination of the reaction as a whole. However, from previous studies in which the Arthus vascular inflammation was prevented by depletion of polymorphs(2), few cellular thromboses occurred indicating that thrombosis comes about secondary to the inflammatory reaction of the vessels. Moreover, that the thromboses resolve secondary to decreasing vascular inflammation seems most likely.

Summary. 1) Our studies suggest that neither exhaustion of humoral or cellular factors necessary for inflammation, nor presence of possible inhibitors of the reaction play a dominant role in diminution of inflammation and healing of a hypersensitivity vasculitis of the Arthus type. 2) This study supports the importance of ridding the diseased vessel of antigen and antibody in bringing about a decrease in reaction and healing.

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Lactogenic Hormone Requirements for Milk Secretion in Intact Lactating Rats.* (25066)

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Early studies indicated that lactogenic hormone released from the adenohypophysis in response to nursing stimuli plays an important role in maintenance of lactation(1,2,5). Recent experiments have indicated that lactogenic hormone is rather ineffective in maintaining milk secretion in hypophysectomized

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rats but when combined with other hormones about 50% of normal milk production was obtained (3,4). The possibility exists, as a result of these investigations, that lactogenic hormone is effective in maintaining lactation only when metabolic requirements for milk secretion have been met. It seemed of interest, therefore, to determine if a more accurate quantitative assessment of loctogen requirement for milk secretion could be made by using intact animals whose general metabolism was unimpaired.

Materials and methods. Adult primiparous lactating rats of Sprague Dawley-Rolfsmeyer strain were housed in individual cages in a room maintained at 78 ± 1°F and given free access to Purina Lab. Chow and water. Each litter was adjusted shortly after birth to 6 young. On day 14 postpartum the following procedure was employed to estimate amount of milk secreted by a lactating rat during 2 consecutive 12-hour periods: Each litter was isolated from their mother during each 12-hour period. At onset of first isolation, 3 additional young of comparable size and weight from donor mothers were added to each litter of 6. *Control.* Shortly after end of first isolation period each of 14 lactating rats was weighed to nearest .5 g and injected subc. with 1 unit oxytocin.‡ The young were immediately replaced and allowed to nurse. A second injection of 1 unit oxytocin was administered 10 minutes later. After the young had nursed a total of 30 minutes, each mother was reweighed. The same procedure then was repeated after a 2nd 12-hour period of isolation of mother and young. *Experimental.* Fifty-six lactating rats were injected i.p. with 4.5 mg/100 g Nembutal 20 minutes prior to end of first isolation period. A depth of anaesthesia was reached within 20 minutes whereby breathing became deep and regular and spinal reflexes were obtunded, *e.g.*, lack of flexor response when Achilles tendon or feet were pinched tightly. Each mother was weighed to nearest .5 g, laid on her side and her young replaced. One unit oxytocin was injected i.p. a few minutes after the young became attached to the teats. A second injection of 1 unit oxytocin was administered 10 minutes later. When it became apparent from behavior of the young that milk flow from mammary glands had ceased, the litter was removed and each mother reweighed. Five lactating rats were similarly treated except 2.5 mg/100 g Nembutal was administered, a level which resulted in loss of consciousness but without abolishment of spinal reflexes. Immediately following completion of nursing, mothers received a single subcutaneous injection of .2 ml saline or .5, 1, 1.5 or 2 mg lactogenic hormone§ each in .2 ml saline. In one group, however, a second injection of hormone was made 6 hours after the first. Following a 2nd 12-hour isolation of mother and young, each lactating rat was placed under light Nembutal anaesthesia and milked out as described for first nursing.

Difference in maternal body weight before and after each nursing was used to represent amount of milk secreted during previous 12 hours. All rats were examined at end of first nursing to ascertain whether all glands had been nursed. If one or more glands were not nursed the teats were covered with Scotch tape prior to 2nd nursing. In such cases amount of milk was calculated for 12 glands on basis of that obtained from nursed glands. All lactating rats were killed after 2nd nursing, skinned and their mammary glands examined for presence of milk. In a preliminary study 10 lactating rats were killed at completion of the first nursing and their mammary glands similarly examined.

Results. The technic of injecting 1 unit oxytocin 2 × at 10 minute intervals to conscious or anaesthetized lactating rats on day 14 postpartum, and using 9 young to nurse the glands, proved to be an effective means of evacuating almost all of milk which had been secreted during a previous 12 hour period. Gross examination of 10 rats after the first nursing in a preliminary experiment, and of all animals in the present study after the second nursing showed the mammary glands to be essentially devoid of milk. They were quite red in appearance with only an occasional splotch of white. Weight loss of moth-

‡ Kindly supplied by Armour Labs.: diluted to contain 1 unit/.2 ml.

§ Lactogenic hormone was a gift from Endocrinology Study Section, N.I.H.

TABLE I. Effect of Lactogenic Hormone upon Milk Secretion by Lactating Rats on Day 14 Postpartum. Lactogen administered after 1st nursing.

Treatment	No. of rats	1st nursing: Prenursing body wt (g)	1st nursing: Milk* (g)	1st nursing: Milk (g/100 g)	2nd nursing: Prenursing body wt (g)	2nd nursing: Milk* (g)	2nd nursing: Milk (g/100 g)	% of control
Control	14	306.9	18.9	6.16 ±.18	297.9	8.7	2.92 ±.19[1]	
Nembutal, 2.5 mg/100 g	5	323.4	20.4	6.30 ±.36	306.0	7.6	2.48 ±.22	
" , 4.5 "	11	301.0	18.9	6.27 ±.24	287.3	2.5	.85 ±.05[2]	29.1
Idem + .5 mg lactogen	10	315.7	19.5	6.18 ±.24	301.5	5.4	1.79 ±.14[3]	61.3
" + 1.0 mg lactogen	10	294.4	18.1	6.15 ±.25	283.1	5.7	2.01 ±.18[4]	68.8
" + 1.0 mg lactogen (.5 mg 2×)	7	302.1	18.1	6.00 ±.15	275.6	5.3	1.92 ±.18[5]	65.8
" + 1.5 mg lactogen	9	301.2	18.7	6.21 ±.22	284.1	5.0	1.76 ±.17[6]	60.3
" + 2.0 " "	9	321.3	20.8	6.47 ±.22	304.2	6.1	2.01 ±.17[7]	68.8
Mean	75	307.2	19.1	6.22 ±.05				

* Milk from 12 mammary glands removed by 9 nursing young with aid of oxytocin (1 unit 2× at 10 min. intervals).

Student's "t"	Probability
1–3,4,5,6,7	.025
2–1,3,4,5,6,7	.001
3–7	.5

ers proved preferable to weight gain by nursling young as an indication of amount of milk present in glands. Defecation or urination did not occur in mothers but invariably occurred in young once milk flow had commenced. Preliminary data also indicated 9 young were more effective than 10-12 for purposes of total milk withdrawal primarily because of less crowding and reduced competition for teats. With this technic an average of 19.1 g of milk was obtained from 75 lactating rats following initial 12 hours allowed for milk secretion. A significant positive correlation ($P = <.01$) existed between amount of milk obtained and maternal body weight. Values obtained thus were expressed as g/100 g body weight. On this basis, milk yield values were very uniform between groups and also between individuals in any one group (Table I).

Milk secreted during second 12-hour period by untreated lactating rats averaged 2.92 g/100 g or 47.2% of that obtained after the 1st 12-hour period (Table I). Administration of 2.5 mg/100 g Nembutal at time of first nursing had no effect upon amount of milk secreted during 2nd 12 hours. Rats injected with 4.5 mg/100 g Nembutal, a level which resulted in deep anaesthesia for about 1 hour, exhibited a significant reduction in average milk yield of .85 g/100 g following second 12-hour period. No overlap with control values occurred (Table II). Injection of .5, 1.0, 1.5, or 2.0 mg lactogenic hormone/rat at end of first nursing to deeply anaesthetized lactating rats evoked significant restoration ($P = .001$)

TABLE II. Distribution of 2nd Nursing Milk Yield Values from Table I.

Distribution of:	Nembutal range	Avg milk (g/100 g) Control range		
	.7–1.1	1.4–2.3	2.4–3.3	3.4–4.3
Control		4	5	5
Nembutal, 4.5 mg/100 g	11			
Idem + .5 mg lactogen	2	7	1	
" + 1.0 mg "	1	6	3	
" + 1.0 mg " (.5 mg 2×)	2	2	3	
" + 1.5 mg "	1	7	1	
" + 2.0 mg "	2	4	3	
Total lactogen	8	26	11	

in milk secretion (Table I). Progressively greater milk yield did not result from administration of larger doses of lactogen or, in one instance, from dividing the dose and injecting 2 × at 6-hour intervals. Analysis of correlation coefficients indicated, however, that milk secretion resulting from injection of lactogen was significantly greater ($P = .01$) in those rats from which greater milk yields were obtained at first nursing. Average yield obtained from injecting any one level of lactogen was still significantly less ($P = .025$) than control value of 2.92 g/100 g. Administration of any one level of lactogenic hormone was not effective in all instances (Table II). One or 2 rats in each lactogen-treated group failed to secrete milk in excess of range of values obtained for saline-injected anaesthetized group.

Discussion. The role of lactogenic hormone in initiation of lactation is well known. However, the extent to which the hormone maintains or influences intensity of lactation is less clear. It has been suggested that regular application of milking or nursing stimuli are necessary for effective maintenance of lactation since such stimuli evoke rapid release of oxytocin from the neurohypophysis for milk removal(6) and of lactogen from the adenohypophysis for milk secretion(7). The observation that an appropriate depth of anesthesia effectively blocked pituitary discharge of both hormones in response to nursing stimuli in lactating rats(8) provides a technic whereby requirements of each hormone for milk secretion and removal in intact animals can be quantitatively estimated. Estimation of oxytocin requirements with this method has been reported(9). It has been substantiated in the present study that 4.5 mg/100 g Nembutal effectively prevents release of lactogen. Anaesthetized rats whose mammary glands have been emptied of milk with oxytocin failed to secrete appreciable quantities of milk during a subsequent 12 hour period but injection of lactogenic hormone to such rats restored milk secretion approximately 70% of normal. Attempts to maintain milk secretion in hypophysectomized rats with lactogen alone have not met with this degree of success. When combined with various other hormones only about 50% of normal milk production has been obtained(3,4). This suggests that replacement of hypophyseal hormones in the proper ratios has not yet been attained. It has been suggested that lactogen perhaps triggers enzyme systems involved in synthesis of milk from precursor substances(10,11). It would appear, therefore, that a proper balance of hormone influencing the well-being of the organism and regulating the supply of metabolites for milk synthesis must be present for lactogen to be fully effective. The results of the present investigation, in addition to confirming the concept that lactogen is vitally concerned in maintenance of lactation, lead to 2 additional considerations. The observation that lactogen was not equally effective in restoring milk secretion in lactating rats suggests that amount of lactogen reflexly released from the pituitary in response to nursing stimuli may limit the intensity of milk secretion in some animals. It follows that supplemental administration of hormone might improve lactation in such cases. The second consideration stems from inability of lactogen alone to produce 100% restoration of milk secretion. The possibility exists that Nembutal blocks release of other anterior pituitary hormones which normally might be reflexly released by stimulus of nursing and which might act synergistically with lactogen to effect full milk secretion. There is evidence that nursing stimuli during lactation induces release of ACTH(12,13) and it is quite possible growth and thyrotropic hormones are similarly affected.

Summary. A technic is described for determination of amount of lactogenic hormone required for milk secretion in intact lactating rats. Rats were isolated from their litters for 12 hours on day 14 postpartum then deeply anaesthetized with 4.5 mg/100 g Nembutal and all milk withdrawn by 9 young with aid of oxytocin. With lactogenic hormone discharge blocked and mammary glands empty, significantly less milk (.85 g/100 g maternal body weight) was obtained following a 2nd 12-hour isolation period in comparison with 2.92 g/100 g for unanaesthetized controls. Injection of .5, 1, 1.5 or 2 mg lactogenic hormone during 2nd isolation period significantly restored milk yield approximately 70%. Yields

were, nevertheless, significantly less than controls. No greater milk yield resulted from injection of more than 1 mg lactogen. These data indicate that lactogen is necessary for maintenance of milk secretion in intact rats and strongly imply that since lactogen alone was incapable of restoring milk secretion 100%, other pituitary hormones are necessary for full milk secretion and perhaps might be susceptible to discharge by nursing stimuli.

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Excretion, Enterohepatic Circulation, and Retention of Radiovitamin B_{12} in Pernicious Anemia and in Controls.* (25067)

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The routes and patterns of Vit. B_{12} excretion have been studied in animals(1,2) and in man(3,4,5). In human studies, some indications were found of an intestinal reabsorption of Vit. B_{12} excreted in bile. The purpose of the present paper is to confirm this finding and to extend the studies to patients with deficient B_{12}-absorption. Retention of B_{12} in serum, and B_{12} excretion in controls and in patients with B_{12}-deficiency were also studied.

Material and methods. Controls were 11 healthy, non-hospitalized males between 20 and 34 years old (normal controls), and 4 female and 2 male hospital patients between 16 and 69 years old (control patients). Their diagnoses were asthenia and achlorhydria, allergic bronchial asthma, diabetes mellitus, and acute or subacute nephritis (3 cases). Random serum samples showed normal B_{12} concentrations. All control patients had normal Vit. B_{12}-absorption tests (21-55%) by the technic described earlier(5). Four additional patients with a post-operative drain in the common bile duct were employed to obtain several bile samples within a short time. The 13 cases with disturbed B_{12} metabolism (Table I) had either an acute Vit. B_{12} deficiency as evidenced by low Vit. B_{12} levels in serum and by megaloblastic anemia reacting to B_{12} treatment, or disturbed Vit. B_{12}-absorption, mostly both. Case No. 4 is insufficiently studied and results based on material involving this case are in brackets (Table IV). Between 0.3 and 0.6 μg Vit. B_{12} labeled with Co^{56} or Co^{58} and with approximate specific activity of 2 mc/mg were injected intramuscularly. Two persons received 1 μg because of the decay of radioactivity. Twenty-nine serum samples were obtained from 17 persons between 3-17 days after injection of labeled B_{12}. All urine was collected from 24 persons during 3 days after injection. From 29 persons 136 24-hour feces collections were obtained, starting on the average 6.8 (4-10) days after radiovitamin injection. Feces were liquefied by nitric acid digestion and concen-

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