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Effect of Pregnancy and Lactation on Granuloma Tissue Formation and Joint Permeability in Rats.* (20733)

ROLAND K. MEYER, JACOB C. STUCKI, AND K. A. AULSEBROOK.
From the Department of Zoology, University of Wisconsin, Madison.

Hench confirmed and extended the general observation that pregnancy has a beneficial effect on rheumatoid arthritis in the human female(1). Subsequently he and his coworkers administered large doses of ACTH or cortisone to patients with rheumatoid arthritis and obtained a dramatic and prompt improvement(2). These results made it seem probable that adrenal cortical hormones of the gluco-corticoid type are the endocrine agents responsible for the improvement seen during pregnancy and stresses, although as Selye states, "the beneficial effect of pregnancy upon rheumatoid arthritis has repeatedly been confirmed, but the underlying mechanism is still obscure(3)."

In view of the complex endocrine mechanisms existing during pregnancy and their relationship to arthritis, it became of interest to make a quantitative study of the effect of pregnancy and lactation in the rat on connective tissue formation induced by the subcutaneous implantation of cotton pellets, and on the permeability of knee joint synovial membranes injured by the intra-joint injection of formaldehyde.

Materials and methods. Female rats 90-120 days of age were obtained from Sprague-Dawley, Inc., and maintained on Rockland Rat Diet. Males were placed with the females in the evening and vaginal smears were taken in the morning to detect sperm. The day on which sperm were found was considered as day 0 and the next day as day 1 after insemination. Lactation was standardized by maintaining the number of sucklings at 8 throughout the period of lactation. Cotton pellets weighing between 4-5 mg were selected from those supplied to the dental profession in package form. Each pellet was implanted through a small incision into the loose subcutaneous connective tissue according to the method reported by Meier *et al.*(4) and modified in our laboratory by Shipley and Meyer (5) who have shown that systemically or locally administered gluco-corticoids inhibit the formation of granuloma tissue around the implanted pellets. Care was taken to place the pellet in the subcutaneous pocket in such a way that connective tissues surrounded it on all sides. The skin incisions were closed with skin clips. Pellets were bilaterally implanted in both the pectoral and groin regions in one experimental series and in another experiment in the dorso-lateral neck region only. The pellets were implanted into rats on the day of insemination and on days 6, 12, 18, 24, 30, 36, and 42 after insemination. The pellets and their attendant granulation tissue (granulomas) were removed after having been in place for 120 $\pm$ 4 hours. Any one rat carried pellets during one stage of pregnancy or lactation only, and no pellet was in place more than 120 $\pm$ 4 hours. Control animals were non-pregnant female rats which were having regular estrous cycles and were implanted with pellets without regard to stage of cycle at the time of implantation. After the pellets had been in place for 5 days the rat was killed and each granuloma was dissected out of the surrounding loose connective tissue and weighed on a torsion balance. Granulomas which were

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TABLE I. Weights of Granulomas Induced by Implanted Cotton Pellets and Clearances of Para-Amino Hippurate from Injured Knee Joints of Rats during Pregnancy and Lactation.

Day pellets removed*	Avg granuloma wt ± S.E. (mg)† A	B	Avg PAH recovery ± max dev. from mean (%)
Non-pregnant	65.2 ± 3.8 (6)‡	72.4 ± 3.0 (7)‡	22.2 ± 2.8(16)‡
5	74.8 ± 6.4 (6)	80.0 ± 2.0 (5)	21.6 ± 2.6 (5)
11	62.4 ± 3.5 (8)	69.0 ± 5.8 (4)	28.2 ± 3.0 (6)
17	50.0 ± 1.8 (5)	59.3 ± 2.5 (4)	41.4 ± 4.1 (6)
23	51.0 ± 2.8 (4)	50.8 ± 3.1 (5)	39.0 ± 2.2 (5)
29	63.5 ± 3.1 (4)	70.0 ± 4.5 (5)	20.8 ± 4.9 (6)
35	53.4 ± 1.7 (5)	57.4 ± 5.0 (5)	23.1 ± 2.6 (5)
41	57.0 ± 2.6 (3)	58.8 ± 4.9 (5)	19.7 ± 3.3 (5)
47	46.6 ± 2.4 (5)	63.0 ± 4.0 (2)	29.3 ± 1.1 (5)

* Day after insemination.
† Includes fluid absorbed by cotton but not cotton itself.
‡ No. of rats.
A = Pellets in pectoral and groin regions; 4 pellets/rat.
B = " " neck region; 2 pellets/rat.

obviously infected were discarded. Pregnancy was confirmed at the time of autopsy. The value for the average net granuloma weight in any group of rats was determined as follows: The initial dry weight of the cotton pellet was subtracted from the total wet weight of each granuloma removed, giving the net weight, which included the fluid absorbed by the cotton. The average net weight of the granulomas from each rat was determined, and the mean of these averages ± the standard error of the mean was calculated for each group. The method used to determine the permeability of the synovialis was developed in the rat by Aulsebrook and Meyer(6). They have shown that the intra-joint injection of minute amounts of formaldehyde decreases the clearance of sodium para-amino hippurate (PAH) which is injected into the joint cavity 18-20 hours after the administration of formaldehyde. Cortisone or cortisone acetate administered 24 hours before and 6 hours after the injection of formaldehyde prevents the decrease in the permeability and maintains the normal clearance rate of PAH.

Some of the animals carrying pellets and other comparable rats not implanted with pellets were prepared for the joint permeability tests by injecting 0.004 cc of a 1.0% solution of formaldehyde into the knee joint approximately 20 hours before the animals were killed for the removal of granulomas. The quantity of dye excreted during the 30-minute period is expressed as percentage recovery of the quantity injected into the joint cavity. The values for each group of rats are presented as the mean ± the maximum deviation from the mean observed for the group.

Results and discussion. The data for the formation of connective tissue induced by cotton pellets are tabulated in Table I, and graphically presented in Fig. 1. The data in column A, Table I, are from the experiment in which pellets were implanted in the pectoral and groin regions, 2 per region, a total of 4 per rat. It was noted during the autopsy of these rats that as pregnancy advanced the developing mammary glands extended into the regions where the pellets were implanted. This condition suggested that the decreased weight of the granulomas observed after the 11th day of pregnancy might be due to an interference in the development of connective tissue by the mammary glands. In a subsequent experiment, pellets were implanted into the dorso-lateral neck region, one on each side, 2 per rat. The values for this series are presented in column B, Table I.

A study of the values for the wet weights of the granulomas removed during pregnancy and lactation in Exp. A and B shows that granulomas removed on the 5th day of pregnancy are slightly heavier than those obtained from non-pregnant animals. After the 5th day the weight of the granulomas formed during each 5-day period decreases progressively up to about the time of parturition. The values obtained on the 23rd day are significantly lower than those obtained on the 5th day, P = .03 (A) and .0001 (B), or from

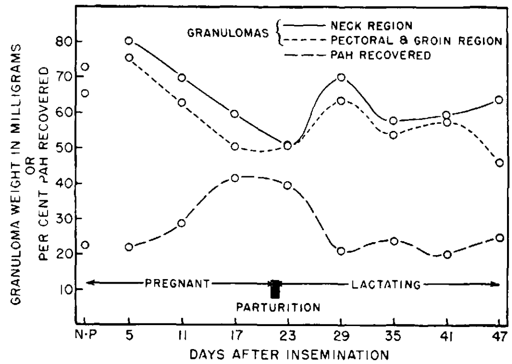

FIG. 1. Weights of granulomas induced by sub cutaneous implantation of cotton pellets and clearances of sodium para-amino hippurate from formaldehyde injured knee joints of rats during pregnancy and lactation.

non-pregnant rats, P = .03 (A) and .001 (B). Between the 23rd and 29th days post-insemination there is a significant increase in weight, P = .03 (A) and .01 (B), the values obtained on the 29th day approximating those found for non-pregnant animals. Between the 29th and 35th days post-insemination there is a decrease in values. These tend to remain low throughout the remainder of lactation. The granuloma weight decrease from the 29th to the 35th day is significant only in Exp. A where pellets were implanted in the pectoral and groin regions, P = .03 (A) and .10 (B), but the 35-day values in both Exp. A and B are significantly lower than those obtained from rats on the 5th day post-insemination, P = .02 (A) and .005 (B), and from non-pregnant rats, P = .03 (A) and .03 (B).

The weight of the relatively light granulomas obtained on the 23rd day post-insemination represents largely, we believe, fluid absorbed by the pellets very shortly after implantation, rather than tissue. In experiments which were designed to study this problem we have found that pellets of the weight used in this study soak up approximately 30 to 40 mg of fluid within 3 hours after implantation. This represents about the maximum capacity of a pellet of this weight to absorb fluid. The weight of the granulomas on the 23rd day is only 20 to 40% more than pellets holding a maximum amount of fluid, while those obtained on the 5th day weigh from 90 to 160% more than saturated pellets. The weight of the granulomas removed on the 23rd day is of the order of those found in our laboratory by Shipley and Meyer(5) for adrenalectomized rats implanted with pellets and given cortisone, hydrocortisone, 11-dehydrocorticosterone or corticosterone. Their data are similar to those reported earlier for cortisone by Meier *et al.*(4) who used cotton pellets, and by Taubenhaus and associates(7,8) who induced granulation tissues by the use of turpentine. Other investigators(9-12), utilizing other criteria, have also demonstrated that connective tissue formation is markedly inhibited by cortisone and ACTH.

It is of interest at this point to correlate the changes in the clearance of PAH from the formaldehyde injured joints of the pregnant and lactating rats with the weights of the granulomas. As the data in Table I show, the clearance values are low in non-pregnant females having regular estrous cycles and in rats on the 5th day post-insemination. After the 5th day the values progressively increase, reaching a maximum on the 17th day. Beginning just after parturition the clearance of PAH rapidly diminishes reaching early pregnancy values on the 29th day post-insemination. During the remainder of lactation the clearance values are low and show little variation from those obtained shortly after parturition.

The clearance rates observed on the 5th and 11th days are similar to those obtained by Aulsebrook and Meyer(6) in intact non-pregnant female rats in which the knee joint synovial membrane has been injured by the intrajoint injection of formaldehyde. The clearances during the period extending from late pregnancy through parturition are essentially like those found by the same authors for non-pregnant rats given cortisone just before and just after their knee joint synovial membranes had been injured by formaldehyde, or for non-pregnant rats with uninjured joints. They concluded that the injurious effect of formaldehyde, which is reflected by the impaired clearance of PAH from the joint, is prevented by cortisone.

On the basis of evidence from the literature, which is admittedly largely from studies made on the human female(13-16), it is suggested that the inhibition of granuloma formation and the prevention of the injurious effect of formaldehyde on the synovialis in the pregnant rat is due primarily to high levels of glucocorticoids existing during pregnancy. It is further suggested that the source of the steroids is either, 1) maternal adrenals alone responding to the stress of pregnancy(17) and/or 2) maternal and fetal adrenals(18,19) (especially during later stages of pregnancy) and the placentas. The larger granulomas and the smaller clearance of PAH seen during the first week of lactation might be due to the absence of that part of the pregnancy corticoid secretion produced by the fetal adrenals and/or placentas, and to the failure of the maternal adrenals during this stage of lactation to secrete corticoids in quantities equal to those existing in late pregnancy. The smaller granulomas obtained during the remainder of lactation indicate a subsequent increase in the production of corticoids by the maternal adrenals. It is suggested, however, on the basis of the low clearance of PAH during this stage, that the level of corticoids is still less than that existing during the later part of pregnancy.

The ovary of the rat, especially the corpora lutea, may also produce anti-inflammatory substances during lactation as well as pregnancy. Preliminary data obtained in our laboratory with the cotton pellet method, however, indicate that neither progesterone, estrone, nor estradiol in amounts believed to be greater than those found during pregnancy and lactation, inhibit the formation of granulation tissue in hypophysectomized or adrenalectomized rats. Others using intact animals have obtained data which indicate that estrogens prevent the growth of connective tissue (20), and still others that they do not(21).

It may be that the ovarian steroids are part of a unique complex of interacting synergisms and inhibitions between gonadal, adrenal and placental steroids. This complex constitutes an important part of the environment in which reacting enzyme and substrate systems make for the unique biochemical pattern which characterizes the pregnant state. At the same time this complex provides a regulatory mechanism which minimizes the potential injury to the maternal cells which might result from the unopposed action of any one of the endocrine components. We have studies in progress which may yield data that contribute to our understanding of the role of steroidal complexes in regulating the mechanisms involved in resistance to stresses.

Summary. 1. Pregnancy and lactation in the rat markedly influenced the formation of granuloma tissue induced by subcutaneously implanted cotton pellets. During pregnancy there was a progressive reduction in the amount of granuloma tissue formed, the least amount being formed at about the time of parturition. After parturition there is a pronounced increase, followed by a reduction to values approximately like those obtained during late pregnancy. 2. The injurious effect of formaldehyde on the synovialis of the knee joint as measured by the clearance of sodium para-amino hippurate (PAH) is likewise progressively decreased as pregnancy proceeds, injury being almost completely absent at about the time of parturition. After parturition the PAH clearance quickly returned to the non-pregnant or early pregnancy levels. Lactation did not provide the synovialis with any measurable protection against formaldehyde. 3. The inhibition of granuloma formation and the protection of the synovialis against the injurious effects of formaldehyde are believed to be due primarily to an increase in the gluco-corticoid moiety of the unique complex of interacting steroids existing during pregnancy especially, and to a lesser extent during lactation.

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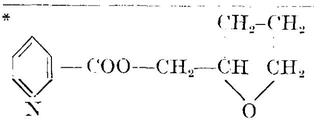
A Cutaneous Test for Rheumatic Activity in Children. (20734)

MURRAY M. STREITFELD AND MILTON S. SASLAW.
(Introduced by Robert C. Batterman.)
From the Department of Medical Research, National Children's Cardiac Hospital, Miami, Fla.

The evaluation of activity of rheumatic fever is made clinically on the basis of Jones'(1) criteria and the combined results of such tests as antistreptolysin-O, antistreptokinase, antihyaluronidase, and C-reactive protein determinations(2-6). No single test permits estimation of rheumatic status. Our studies directed toward finding more adequate diagnostic aids led us to the work of Nassim and Banner(7), who described the absence of a cutaneous hyperemic and/or edematous response to the topical application of a 5% water-miscible cream containing the rubefacient(8-11), tetrahydrofurfuryl ester of nicotinic acid, in 5 patients with active rheumatoid arthritis. It appeared to us that the skin response to this drug might be of diagnostic value in rheumatic fever.

Materials and methods. The skin response to topical application of a vaseline-lanolin ointment containing 5% tetrahydrofurfuryl ester of nicotinic acid* was determined in 337 white and Negro children, several months to 18 years of age.† All skin tests were performed and interpreted by the authors without knowledge of the clinical diagnoses and estimates of rheumatic activity, which were made independently by the clinical or medical director of each institution. Thus the tests were done "blindly", those observing and interpreting the responses being completely unaware of the probable diagnosis, and the clinicians being unaware of the test results. Seventy mg of the nicotinic acid ester ointment was gently rubbed, by means of the examiner's finger (covered by finger cot), with 35 circular strokes into an area $1\frac{1}{2}$ inches in diameter on the volar aspect of the forearm about an inch above the wrist. A control ointment was applied in the same manner, either on the forearm above the test area or

* 
Supplied by Ciba Pharmaceutical Products, Summit, N. J., under trade name Trafuril.

† These studies were carried out at our hospital, Children's Heart Hospital (Philadelphia, Pa.), Gallinger Municipal Hospital (Washington, D.C.), Irvington House (Irvington-on-Hudson, N. Y.), Jackson Memorial Hospital (Miami, Fla.), and King's County Hospital (Brooklyn, N. Y.). The authors acknowledge with thanks the co-operation of Drs. Francisco A. Hernandez, Gene H. Stollerman, Ella Roberts, Joseph Fazekas, Richard S. Gubner, Franz H. Stewart, Scheffel H. Wright and Warren W. Quillian, and the medical and nursing staffs of these institutions.