PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 152, 549-553 (1976)

Effect of Skin or Muscle Incisions or the Loss of Blood on Serum Leucogenenol Level[1] (39437)

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Leucogenenol, originally isolated from the metabolic products of *Penicillium gilmanii* (1), and demonstrated to be 2-(1,2-dihydroxy-3-methyl-5-oxocyclohexyl)-3,11-dihydroxy-11-(hydroxymethyl)-9-methyl-1-oxa-5-azaspiro [5.5] undeca-2,4-dien-7-one (2,3) is normally present and was isolated in concentrations of approximately 5 mg/kg (dry weight) from liver (4), thyroid, thymus, and gonads (5), and in concentrations of 0.06 mg/kg (human) to 0.6 mg/kg (bovine) from blood serum (6) where it is associated with a carrier protein (7).

Although leucogenenol has no significant effect on the rate of replication of or uptake of tritiated thymidine by lymphoblastoid cells in tissue culture, it does affect their oxygen uptake and carbon dioxide evolution and thus, their respiratory quotient (8–10). Under well-defined conditions, an optimum quantity of leucogenenol (1.9×10^{-4} μg/approx 10^5 cells) causes an easily measurable change in respiratory quotient (ΔRQ) of L5178Y cells of from 1.0 to 1.7. Greater or lesser than optimum quantities of leucogenenol have a significantly less effect on the ΔRQ (10) and the ΔRQ is related to the log of the reciprocal of the quantity of leucogenenol added to the medium by a gaussian type mathematical function. The latter observation made possible the development of an assay (6) in which the quantitative isolation of leucogenenol from a known volume of serum is carried to a stage where no compounds are present other than leucogenenol that affect the ΔRQ of L5178Y cells. The concentration of leucogenenol in the serum is then calculated from the dilution of a known volume of a solution of this partially isolated leucogenenol that causes a maximum ΔRQ of L5178Y cells. When known quantities of leucogenenol are added to samples of sera these quantities are accurately determined (within 5%) by the bioassay.

The concentration of leucogenenol in the serum varies with the species of animal (5,6) and in particular with abnormal conditions (11). A preliminary investigation of the levels of leucogenenol in the serum of patients with a variety of diseases showed that it is not significantly elevated in noninflammatory diseases but is significantly elevated in patients with diseases that are associated with tissue changes or destruction[2] such as systemic lupus erythematosus, rheumatoid arthritis, or subacute bacterial endocarditis.

Injection of leucogenenol into animals results in an increase in the rate of maturation of the myeloblasts and erythroblasts in their bone marrow (12–17), and addition of leucogenenol to a tissue culture of human peripheral lymphocytes induces blast formation (18). It is apparent that if leucogenenol also affects the rate of transformation and/or maturation of cells associated with tissue repair an elevation in serum leucogenenol might be expected in diseases associated with tissue destruction. It might also be predicted that artificially induced loss of, or injury to tissues of normal animals should result in elevated levels of leucogenenol in their sera.

We wish to report that skin or muscle incisions or the loss of appreciable quantities of blood result in the temporary elevation of the level of leucogenenol in the sera of rabbits and rats.

Materials and methods. Rabbits (New Zealand whites of 3 to 5 kg in weight) and rats (Sprague-Dawley, approximately 250 g in weight) were exposed to a light and dark cycle of 12 hr each, and allowed food (Pur-

[1] Supported in part by Office of Naval Research Contract No. N00014-73-C-0033, and National Institutes of Health Grant No. CA12736-02.

[2] M. B. Stevens, F. A. H. Rice, J. D. McCurdy, and C. G. Chen, unpublished data.

ina Lab Rabbit or Rat Chow, as appropriate) and water *ad libitum*. Ether (Mallinckrodt, anesthesia grade) was used for anesthesia. Blood was drawn with sterile disposable syringes and the serum was obtained after allowing the blood to clot for approximately 1 hr at room temperature and overnight at 4°. The serum was removed with a disposable Pasteur pipet. The concentration of leucogenenol in the serum was determined as previously reported (5, 6).

Effect of a skin incision on the concentration of leucogenenol in the serum of rabbits. Two to three milliliters of blood was withdrawn from the marginal ear vein of each of 16 rabbits (8 males and 8 females). The serum was separated and assayed for its concentration of leucogenenol. At random intervals over a period of 3 weeks, blood was again withdrawn (altogether 42 samples) one or more times from the marginal ear vein of each rabbit, the serum was separated and assayed for its concentration of leucogenenol. Eight of the 16 rabbits (4 males and 4 females) then were selected at random, anesthesized, and 2 to 3 ml of blood was withdrawn from the marginal ear vein of each rabbit, the serum was separated and assayed for its leucogenenol concentration. One week later the eight rabbits were anesthesized again and an incision (approximately 10 cm) was made through the skin on the back of each rabbit midway between the neck and the tail. The incision was immediately closed with silk sutures and anesthesia discontinued. At intervals after closing the incision (1/4 to 1/2 hr, 1 hr, 3 hr, 5 hr, 24 hr, 3 days, and 6 days) 2 to 3 ml of blood was withdrawn from the marginal ear vein of three or more of the rabbits, the serum was separated and assayed for its concentration of leucogenenol.

Effect of the loss of blood on the concentration of leucogenenol in the serum of rabbits. Six rabbits (3 males and 3 females) were selected at random from the remaining eight rabbits and 25 to 30 ml of blood withdrawn from each rabbit by cardiac puncture under anesthesia. The serum was separated and assayed for its concentration of leucogenenol. Twenty-four hours and again 3 days later, approximately 5 ml of blood was obtained from each of the rabbits by cardiac puncture under anesthesia. The serum was separated and assayed for its concentration of leucogenenol.

Effect of skin and muscle incisions on the concentration of leucogenenol in the serum of rats. Twelve male and 12 female rats were exsanguinated (approximately 10 ml of blood) by cardiac puncture under anesthesia. The serum was separated from the blood of each rat and assayed for its concentration of leucogenenol. Twenty-eight male rats then were anesthesized and a right and left lateral rectus incision (approximately 2 cm) was made approximately 2 cm from the midline through the skin and muscles into the abdominal cavity of each animal. The muscle layers were closed immediately with gut sutures and the skin was closed with autoclips (Clay Adams, 9-mm No. B-2355/S, stainless steel). Anesthesia was discontinued then in all but seven rats which were exsanguinated immediately by cardiac puncture and the serum was separated and assayed for its concentration of leucogenenol. Five hours, 24 hr, and 3 days, after closing the incisions, seven of the remaining 21 animals were exsanguinated under anesthesia and the serum separated from their blood and assayed for its concentration of leucogenenol.

Effect of loss of blood on the concentration of leucogenenol in the serum of rats. Blood (approximately 2.5 ml) was withdrawn by cardiac puncture under anesthesia from each of six male and six female rats. The serum was separated and assayed for its concentration of leucogenenol. Blood (approximately 2.5 ml) was withdrawn again by cardiac puncture under anesthesia from each of the male rats at the following intervals after the first withdrawal: 1 hr, 1 day, 3 days, and 6 days. Blood (approximately 2.5 ml) was withdrawn from each of the female rats at the following intervals after the first withdrawal: 1, 3, and 6 days. As before, the serum was separated from the blood of each rat and assayed for its concentration of leucogenenol.

Results and discussion. Table I shows the effect of making a skin incision on the concentration of leucogenenol in the serum of rabbits. Normal male and female rabbits show no significant differences in the con-

TABLE I. EFFECT OF MAKING A SKIN INCISION[a] ON THE CONCENTRATION OF LEUCOGENENOL IN THE SERUM OF RABBITS.

	Concentration of leucogenenol in the serum at intervals following a skin incision (μg/liter)							
Rabbit No.[b]	0	1/4 to 1/2 hr	1 hr	3 hr	5 hr	24 hr	3 days	6 days
Males								
1	55.6	54.4		106.1	60.5	101.0	132.3	50.4
2	55.0	58.8	82.5	73.9	47.6	98.8		52.8
3	59.5		50.7	63.5	63.5	67.5		62.5
4	48.4		23.7	30.2	99.3	214.7	73.6	39.4
Females								
5	38.8	62.5	52.5	99.3	64.1	122.6	68.9	31.4
6	39.9			56.8	28.9	107.8	97.0	26.1
7	65.3				70.5	348.4		53.7
8	58.9				33.6	84.3	62.1	58.9
Mean	52.7	58.2	52.3	71.6	58.5	143.2	75.4	46.9
±S.D.[c]	±9.5		±24	±28	±22	±94[d]	±15[d]	±13

[a] An approximately 10 cm incision was made on the back of each rabbit midway between the neck and the tail. The incision was closed immediately with sutures. For details see text.

[b] Serum obtained at random two to four times over a 3 week period from eight male and eight female rabbits showed a mean concentration of leucogenenol of 50.8 ± 14 μg/liter (42 determinations).

[c] Standard deviation.

[d] $p < 0.001$ for difference from animals before the incision was made.

centration of leucogenenol in their serum (48.4 to 59.5 and 38.8 to 65.3 μg/liter, respectively). Neither is there a significant difference between the concentration of leucogenenol in the blood of unanesthesized animals and animals anesthesized with ether (50.4 ± 14 and 52.7 ± 9.5 μg/liter, respectively). Although 1/4 to 1/2, 1, 3, and 5 hr after making the skin incision the average concentration of leucogenenol in the serum of the rabbit is increased, the variation between animals, as indicated by the standard deviation, makes it impossible to determine whether or not it is significant. However, 24 hr after making the skin incision there is an unquestionable elevation in the concentration of leucogenenol in the rabbit's serum (143.2 ± 94 μg/liter vs the normal 52.7 ± 9 μg/liter). The concentration of leucogenenol in the serum remains elevated for at least 3 days, but returns to a normal value in 6 days.

Table II shows the effect of making two incisions into the abdominal cavity of rats on the concentration of leucogenenol in their serum. No significant change is observed 5 hr after making the incisions. However, the concentration of leucogenenol in the serum is elevated in 24 hr, but decreases to normal values in 3 days.

Table III shows that the concentration of leucogenenol in the serum of both male and female rats and rabbits is elevated the day after the rat has lost approximately one-quarter or the rabbit approximately one-half of the blood in its circulation. A second loss of the same quantity of blood from the rat the day following the initial loss results in the maintenance of the increased level of leucogenenol for at least 2 days. A third loss of approximately the same quantity of blood causes a further mean increase in the concentration of leucogenenol in the rat's serum.

TABLE II. EFFECT OF MAKING TWO INCISIONS[a] INTO THE ABDOMINAL CAVITY OF RATS[b] ON THE CONCENTRATION OF LEUCOGENENOL IN THEIR SERUM.

Concentration of leucogenenol in the serum at intervals after closing the incisions (μg/liter ± standard deviation[c])			
0	5 hr	1 day	3 days
34.9 ± 3[b]	32.1 ± 11	75.0 ± 27[d]	33.8 ± 8

[a] Two approximately 2.5 cm lateral rectus incisions were made approximately 2 cm from each side of the median line and immediately closed with gut sutures (muscle layers) and autoclips (skin). For details see text.

[b] Male rats were used. An additional 12 male and 12 female rats showed normal concentrations of leucogenenol in their serum of 36.9 ± 9 μg/liter.

[c] Calculated from results on seven animals.

[d] $p < 0.001$ for difference from normal animals.

TABLE III. EFFECT OF THE LOSS OF BLOOD ON THE CONCENTRATION OF LEUCOGENENOL IN THE SERUM OF RABBITS AND RATS.

Animal	Concentration of leucogenenol in the serum at intervals following initial or repeated loss of blood[a] (μg/liter ± standard deviation)				
	0	1 hr	1 day	3 days	6 days
Rabbits[a]	50.8 ± 14		162.2 ± 66[d]	76 ± 14	
Rats[b] (male)	32.0 ± 3	46.5 ± 8	96.6 ± 25[c, d]	109.0 ± 51[c, d]	278.4 ± 146[d]
Rats[b] (female)	37.8 ± 9		86.2 ± 25[c, d]	70.6 ± 28[c, d]	250.0 ± 90[d]

[a] Approximately 25 ml of blood was withdrawn by cardiac puncture under ether anesthesia from each of three male and three female rabbits. One day and 3 days later approximately 2.5 ml of blood was obtained from each of the rabbits by cardiac puncture under ether anesthesia. The serum was separated from each sample of blood and assayed for its concentration of leucogenenol. For details see text.

[b] Approximately 2.5 ml of blood was withdrawn from each of six male and six female rats by cardiac puncture under ether anesthesia at each of the indicated time intervals. The serum was separated from each sample of blood and assayed for its concentration of leucogenenol.

[c] Approximately 2.5 ml of blood again withdrawn.

[d] $p < 0.001$ for difference from normal animals.

So far, most studies of the biological activity of leucogenenol have been concerned with its effect on the rate of maturation and/or transformation of blood cells (12–18) or associated phenomena such as antibody formation (19–22). However, there is no reason to conclude that leucogenenol does not have activity towards other cells as well. Most steroid hormones have more than one target organ (23) and growth hormone is not only necessary for general body growth but also is necessary for normal antibody formation (24).

Certainly it is likely to be more than a mere coincidence that damage done to tissues by skin or muscle incisions, ionizing radiation (11), and many inflammatory diseases[2] results in the elevation of the serum leucogenenol level. Fumarola and coworkers have demonstrated that leucogenenol induces a transformation of fibroblasts cultivated *in vitro* (25) and increases the rate at which colloidal carbon is cleared from the circulation of rabbits and rats (26). At least one should consider the possibility that an increased level of serum leucogenenol is a normal physiological response to many types of tissue damage, and that this elevation induces a more rapid formation of appropriate cells for repair and the clearance of debris.

Summary. It has been found that damage to a tissue of a rabbit or a rat, such as results from a skin incision or an incision through the skin and muscles into the abdominal cavity, is followed 24 hr later by a significant increase in the concentration of leucogenenol in the animal's serum. Likewise, loss of approximately one-quarter to one-half of the blood in the circulation of rabbits or rats causes an increase 24 hr later in the animals' serum leucogenenol concentration.

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Received June 2, 1975. P.S.E.B.M. 1976, Vol. 152.