Table 2. Inhibitory effect of arthritic serum on Concanavalin A stimulated ^{3}H-thymidine incorporation in lymph node cells from normal rats: influence of different times of serum addition. 1 µg/ml of Con A and 50 µl of normal rat serum were added at the beginning of the culture, and 50 µl of normal rat serum or arthritic serum were added at the indicated times

Interval (h) between Con A and arthritic serum	Inhibition of ^{3}H-thymidine incorporation (± SD) as percent of control
0	88.2 ± 7.5
1	78.5 ± 10.2
18	6.8 ± 1.1

1 R. F. Mortensen, D. Braun, S. Croft and H. Gewurz, Fed. Proc. *35*, 576, 2047 abs. (1976).
2 R. M. Gorczynski, D. G. Kilburn, R. A. Knight, C. Norbury, D. C. Parker and J. B. Smith, Nature *254*, 141, (1975).
3 A. H. Glasgow, R. B. Nimberg, J. O. Menzoian, I. Saporoschetz, S. R. Cooperband, K. Schmid and J. A. Mannick, New Engl. J. Med. *291*, 1263 (1974).
4 J. G. Levy, A. G. Smith, R. B. Whitney, R. McMaster and D.G. Kilburn, Immunology *30*, 565 (1976).
5 G. Girmann, H. Pees, G. Schwarze and P. G. Scheurlen, Nature *259*, 399 (1976).
6 H. O. Sjøgren, I. Hellstrom, S. C. Bansal and K. E. Hellstrom, Proc. Nat. Acad. Sci. USA *68*, 1372 (1971).
7 R. D. Stout and L. A. Herzenberg, J. exp. Med. *142*, 1041 (1975).
8 B. V. Jegasothy, A. R. Pachner and B. H. Waksman, Fed. Proc. *35*, 575, 2044 abs. (1976).
9 F. D. Wood, C. M. Pearson and A. Tanaka, Int. Arch. Allergy Appl. Immun. *35*, 456 (1969).
10 B. H. Waksman and C. Wennersten, Int. Arch. Allergy *23*, 129 (1963).
11 J. S. Lowe, Biochem. Pharmac. *13*, 633 (1964).
12 H. E. Weimer, F. D. Wood and C. M. Pearson, Can. J. Biochem. *46*, 743 (1968).
13 L. Kourounakis and M. A. Kapusta, Ann. Rheum. Dis. *33*, 185 (1974).
14 A. Kahan, F. Perlik, A. Le Go, F. Delbarre and J. P. Giroud, Agents and Actions *6*, 219 (1976).
15 R. Schmidt-Ullrich, D. F. H. Wallach, Biochem. biophys. Res. Commun. *69*, 1011 (1976).

Con A (figure, D). The basal, non-stimulated ^{3}H-thymidine incorporation was unmodified in all experimental conditions (results not shown).

This inhibitory effect seems to be specifically directed towards the Con A induced mitogenesis since, as shown in table 1, the arthritic serum did not decrease the PHA induced ^{3}H-thymidine incorporation in either normal or arthritic LNC. Moreover, this normal pattern of stimulation with PHA excludes any non-specific action of the arthritic serum on membrane function, thymidine uptake and intracellular thymidine pools.

It is evident from the results reported in table 2 that the inhibition of the Con A stimulated mitogenesis by arthritic serum occurs in the early phases of the process. In fact the ^{3}H-thymidine incorporation was reduced by 88% when the serum was added together with Con A, and by 78% when serum was added 1 h later. No inhibition occurred when the serum was added 18 h after Con A. These observations exclude the possibility of interference of the arthritic serum with binding of Con A to the cell membrane, since this binding is completed within 15 min from the mitogen addition[15].

It should be noted, in addition, that in these experiments only 50% of the serum was arthritic. 50 µl of normal rat serum were added from the beginning of the incubation to allow normal growth until supplementation at the appropriate times with 50 µl of normal or arthritic serum (see 'materials and methods').

The alterations in composition and concentration of the various blood proteins found in adjuvant arthritic rats show a widely different pattern in the different stages of the disease[11,12]. It therefore seems unlikely that variable changes in serum composition may be responsible for an inhibitory effect observed quantitatively unchanged in the different phases of the disease.

The nature of this factor is unknown. It is noteworthy, however, that it is present in the arthritic serum in the very early stages of the disease, before the appearance of the delayed systemic response, and that there is a specific inhibition of the LNC response to Con A. Both Con A and PHA are T-cell mitogens. However, it has recently been reported that the response to Con A is a characteristic of a distinct subpopulation of T-cells isolated from normal mice spleen[7].

Effect of methyl palmitate on the survival of skin semi-allografts in rats

V. Šebestík, E. Paluska, I. Potměšilová and J. Nezvalová

Institute of Haematology and Blood Transfusion, U nemocnice 1, 128 20 Praha 2 (Czechoslovakia),

13 September 1976

Summary. In experiments with rats, the immunosuppressive effect of methyl palmitate on survival of skin semi-allografts was proved. Methyl palmitate was applied in a single dose 1 day prior to transplantation.

Relatively little is known about the inhibitory effect of methyl palmitate (MP) on RES. MP was found to cause lowering of phagocytic activity[1–6] as well as inhibition of primary and secondary antibody response [4–7], while the mechanism of MP action has not yet been fully elucidated. It is not distributed in the organism as other colloids which are phagocyted, and it is quickly hydrolyzed[1]. The effect of MP is manifested mostly in the decreased hepatic and splenic activity of RES, though no substantial damage to these organs could be proved[3,4,6]. According to some authors, the protracted inhibitory

1 D. A. Blickens and N. R. Di Luzio, J. reticuloendot. Soc. *2*, 60 (1965).
2 D. A. Blickens and N. R. Di Luzio, J. reticuloendot. Soc. *2*, 187 (1965).
3 N. R. Di Luzio and D. A. Blickens, J. reticuloendot. Soc. *2*, 250 (1966).
4 N. R. Di Luzio and W. R. Wooles, Am. J. Physiol. *206*, 939 (1964).
5 S. H. Morrow and N. R. Di Luzio, Proc. Soc. exp. Biol. N.Y. *119*, 647 (1965).
6 W. R. Wooles and N. R. Di Luzio, Science *142*, 1078 (1963).
7 V. Šebestík and A. Majský, Folia biol. Praha, in press.

effect of MP on RES can be assumed without subsequent hyperactive phase [3,4]. On the ground of these data, we attempted investigation of a possible immunosuppressive effect of MP on transplantation of a skin graft.

Material and methods. The experiment was performed on 2 groups of rats. The first group was applied MP, the other one was used as control. As skin graft recipients, male rats of Lewis strain, of mean weight 250 g ± 30 g and F 1 (Lewis × AVN) rats as graft donors were used. The skin graft transplantation was done according to Billingham[8]. In thiopental anaesthesia, the donors were excised a graft of 2 × 1,5 cm on the ventral side; the graft was implanted on the dorsal part of the recipient in which dorsal skin in the corresponding extent had been removed. Then the graft was glued in marginal points with colloid, covered with gauze and firmly dressed. The MP emulsion in concentration of 0.5 g MP/ml was prepared as follows: The mixture of MP (Eastman Rochester N.Y.) and 1% of Tween 20 in a 5%-glucose-solution was first homogenized for 10 min in a glass homogenizer and thereafter exposed to sonication for 5 min. The MP particle size oscillated about 1 μm[9]. The pattern of the experiment: 1 day prior to transplantation the experimental group of 23 rats received a single dose of MP emulsion into the tail vein in an amount containing 0.25 g MP per 100 g of body weight. The control group of 16 animals was injected a corresponding amount of Tween 20 in glucose without MP. Following transplantation, the dressings of the controls and experimental group were removed on day 9 and 12, respectively. The statistical evaluation was done by the t-test.

Results and discussion. In the experimental group 3 animals died within 4 h. In the remaining 20 animals and the control group the mean survival time of the graft was 20.3 and 13 days respectively. The difference of the mean survival time of skin grafts is significant at the 0.01 significance level (see table). The study showed that MP applied in a single dose of 0.25 g per 100 g of body weight 1 day before transplantation of the skin graft considerably prolonged its survival. The object of our further work is to establish such a dose, or repeated doses, which would minimize the animals mortality and help to prolong survival of the skin graft.

Survival of skin semi-allografts following application of methyl palmitate

Group	Number of animals	Number of days in survival of grafts In individual rats	Mean	Standard deviation
Experimental with MP	20	25, 22, 20, 20, 20, 21, 21, 26, 18, 18, 18, 18, 20, 20, 20, 24, 17, 20, 19, 19	20.3	2.32
Control (Tween 20)	16	15, 14, 13, 14, 11, 11, 13, 13, 13, 13 11, 15, 13, 13, 13, 13	13.0	1.17

8 R. F. Billingham and W. K. Silvers, in: Transplantation of tissues and cells. The Wistar Institute Press, Philadelphia 1961.
9 V. Šebestík, A. Majský, I. Potměšilová and J. Jelínek, Acta biol. med. germ. *35*, 1379 (1976).

Gamma globulins in the mice vaginal fluids: Cyclic and experimental variations[1]

L. Di Yacovo[2], Clara E. Roig de Vargas-Linares[3] and Angela M. I. Gallardo

Instituto de Histología y Embriología, Facultad de Ciencias Médicas, U. N. C., Casilla de Correo 56, 5500-Mendoza (Argentina), 27 July 1976

Summary. The study of the gamma globulins in the vaginal fluids reveals that the lowest values were found in mice at estrus and in castrated mice 8 days after the estrogen treatment. We suggest that the variation of gamma globulins in the vaginal fluids is influenced both under physiological and experimental conditions by estrogens.

The protein content of secretions bathing the reproductor female tract has been studied by several authors[4-6]. Special attention has been given to the study of the gamma globulins fractions in several secretions in women and in other animal species[7-11]. Previously, cyclical and experimental variations of immunoglobulins of human vaginal fluids have been found, these variations seem to be regulated by estrogen action[12,13]. In the present work we are studying vaginal secretion in mice and their gamma globulins in different physiological and experimental conditions.

320 female albino Swiss virgin mice, 3 or 4 months old, were used. The animals were maintained under the same environmental and nutritional conditions and were divided into 2 groups: I. normal and II. experimental mice. The animals in group I were studied in different cycle conditions: 1. diestrus, 2. proestrus, 3. estrus, 4. metaestrus. By means of the cytological study of the vaginal content, the cyclical condition was determined; thus the animal hormonal status was indirectly appreciated. The animals in group II were castrated by means of a lumbar incision and they were studied 30 days after castration. The vaginal fluids of the same animals were analyzed 3, 8 and 32 days after being treated by a single s.c. injection of 2 μg of 17 B estradiol in oil. The vaginal secretions were obtained washing the vaginal cavity with 100 μl of physiological solution and the cells were removed by centrifugation for 3 min at 12,000 rpm. As the proteins obtained from one animal is too small, pools of 8 or 10 animals were made. The material was lyophilized and then redissolved in 50 μl of physiological solution. Part of the concentrated vaginal secretion was destined to determine total proteins by Lowry et al.[14] method. The other part was studied by agar radial inmunodiffusion method[15] to know the gamma globulin content with relation to a pattern serum (Kallestad lot 012 E 021). Goat antimouse gamma globulins (Kallestad lot 270 F 011) were used in this determination. The gamma globulins