showed a stabilizing effect in arysulfatases and hyaluronidase. However, the result of the administration of vitamin A or glycyrrhizin showed a labilizing effect in both enzymes. A slight labilizing effect in the vitamin A and the hydrocortisone group at 2 weeks after dosing was found in both enzymes (control group: arylsulfatase 9.8 ± 1.5%, hyaluronidase 22.3 ± 5.3%, hydrocortisone group: arylsulfatase 11.5 ± 2.0%, $p < 0.05$, hyaluronidase 29.5 ± 5.0%, $p < 0.01$). However, a stabilizing effect was noticed in the administration group of glycyrrhizin (glycyrrhizin group: arylsulfatase 5.8 ± 1.0%, $p < 0.001$ hyaluronidase 20.3 ± 2.3%, $p < 0.05$).

Discussion. Arylsulfatase and hyaluronidase are lysosomal enzymes. These enzymes are degradation enzymes of the chondroitin sulfates which are components of the connective tissue. It is conventionally known that hydrocortisone is a stabilizing agent, while vitamin A is a labilizing agent. In the present study, a labilizing effect was found at 1 and 2 weeks after the administration of glycyrrhizine and hydrocortisone, however the effects of these agents were reversed at 1 and 2 weeks. In the steroid group, a stabilizing effect at 1 week and a slight labilizing effect at 2 weeks were found; however, in the glycyrrhizine group, a labilizing effect at 1 week and a stabilizing effect at 2 weeks found. These phenomena seem to be attributed to the interaction between the lysosomal membrane and the agent. However, the concrete mechanism was obscure. The amount of hydrocortisone administrated in this experiment was moderate. In clinical treatment, glycyrrhizin used as a curative for chronic hepatitis in spite of the fact that the biochemical mechanism of the drug is still unclear. The administration of glycyrrhizin for a long duration to patients of chronic hepatitis seems to raise the stabilizing effect on the hepatic lysosomes.

6 F. Hutterer, Biochim. Biophys. Acta. *115*, 312 (1966).

Endotoxin-induced acceleration of ovum transport in rabbits[1]

G. Valenzuela, B. J. Hodgson* and M. J. K. Harper

Center for Research and Training in Reproductive Biology and Voluntary Regulation of Fertility, Department of Obstetrics and Gynecology, The University of Texas Health Science Center at San Antonio, San Antonio (Texas 78284, USA),
4 August 1977

Summary. Salmonella enteritidis-Boivin endotoxin (1–20 μg/kg) induced accelerated oviductal ovum transport in rabbits in a dose-related manner. Indomethacin prevented this effect. Levels of prostaglandin E and F in uterine vein blood increased following endotoxin injection.

A drug that reliably accelerated ovum transport through the oviduct and caused premature entry of ova into the uterus would be a useful addition to our contraceptive armamentarium. Appropriate doses of estrogens and progestins can accelerate ovum transport[2]. Prostaglandins (PGs) given after ovulation are also effective, but the response is variable and large doses are required[3–6]. Endotoxin increases the concentration of prostaglandins in cerebrospinal fluid[7], venous blood[8,9] and in urine and uterine endometrial tissue[10].

We have examined the action of endotoxin (*Salmonella enteritidis*-Boivin, Sigma Chemical Co.) on ovum transport in rabbits. Mature female New Zealand white rabbits isolated for 30 days prior to use were injected i.v. with hCG (A.P.L., Ayerst). The rabbits received an injection i.v. of endotoxin dissolved in saline 24 h later and were killed with an overdose of pentobarbital at 48 h after hCG. The genital tracts were removed and flushed to determine the location of ova, and numbers of ovulation points on the ovaries were counted.

In controls killed at 48 h after hCG, 80% of ova are found in the oviduct, but increasing doses of endotoxin from 1 to 10 μg/kg caused a progressive diminution in the percent of ova, in the oviduct, and since few were found in the uterus or vagina were presumably expelled completely from the tract (table 1). The ED_{50} and its 95% probability limits were 3.1 (2.38–4.03) μg/kg and the slope of the dose-response line and its 95% probability limits 5.96 (3.97–8.94).

An additional group of rabbits, similarly treated with 20 μg/kg i.v. at 24 h after hCG, was also given 10 mg/kg i.m. of indomethacin (an inhibitor of prostaglandin synthesis[11]) at the time of endotoxin injection and 4 h later. Indomethacin completely abolished the transport-accelerating effect of endotoxin (table 1).

A group of 6 rabbits was given 100 IU hCG i.v. 22 h later, they were anesthetized with pentobarbital sodium and subjected to mid-ventral laparotomy. 1 uterine vein was catheterized with polyethylene tubing (outside diameter 1.52 mm, internal 0.86 mm). Heparin (1500 units, Upjohn Co.) was injected through the catheter and after a period of stabilization of at least 1 h, a control blood sample was withdrawn. At 24 h after hCG, 20 μg/kg of endotoxin was injected i.v. and blood samples taken into a syringe containing indomethacin (final concentration of more than 10 μg/ml) at various times up to 8 h later. The plasma was separated by centrifugation and frozen at − 20 °C until assay. The thawed samples were extracted with ethyl acetate and separated into 2 portions. Labelled $PGF_{2\alpha}$ or PGE_2 was added to each aliquot and the aliquots redissolved in ethanol were chromatographed on silicic acid columns. Prostaglandins F and E were measured by a double-antibody radio-immunoassay[12–14]. Antibodies to PGF and to PGE were obtained from Miles Laboratories, Inc., Elkhart, Indiana. Each antibody did not discriminate within each class of PGs, but did not exhibit cross-reactivity with other classes of PGs, i.e. antibody to PGEs reacted with PGE_1 and PGE_2 but not with PGFs. The limits of sensitivity for the PGF and PGE radioimmunoassays were 250 pg/ml and 300 pg/ml respectively. Non-specific binding was less than 5% and blank values less than 30 pg. Recoveries, based on labelled $PGF_{2\alpha}$ and PGE_2 added to the samples prior to extraction, ranged from 71.1–100.0% and 51.8–96.0% for the PGF and PGE respectively. A known amount of 2 ng PGF and of 2 ng PGE added to 1 ml of stripped rabbit plasma gave values of 2.14 and 2.20 ng/ml in the assay.

Control values of PGFs and PGEs in uterine vein plasma were relatively low (table 2). Within the 1st h after endo-

toxin, PGF levels did not change while those of PGE increased significantly. At about 90 min PGF concentrations were substantially greater than those of PGE which also continued to increase. Regression analyses showed that PGF and PGE values continued to increase up to approximately 4 h postendotoxin, although the slope of the increase was significantly greater for PGF than PGE. The correlation coefficients were $r = 0.56$ ($p < 0.01$) and $r = 0.75$ ($p < 0.001$) for PGF and PGE respectively. PG concentrations were still elevated 8 h after endotoxin injection.

These results clearly show that endotoxin is associated with increased production of PGs by the genital tract, and accelerates ovum transport.

Endotoxin is clearly as effective as estrogen in accelerating ovum transport. Previous studies with ethinyl estradiol (10 mg/rabbit p.o.), estradiol-17β (250 µg/rabbit i.m.) and estradiol-17β cyclopentylpropionate (250 µg/rabbit i.m.) given at 24 h after hCG have found 15,32 and 8% respectively of ova retained in the oviducts of rabbits at 48 h[15,16]. A dose of 9 mg/rabbit s.c. of $PGF_{2\alpha}$ at 24 h after hCG is required to reduce the percent of ova in the oviduct to 10 at 48 h[17]. In women daily doses of 25 mg of stilbestrol twice a day or of 5 mg of ethinyl estradiol daily p.o. for 5 days are required to ensure a contraceptive effect following unprotected mid-cycle intercourse[18,19]. Such large doses of estrogens cause unwanted adverse reactions.

Endotoxin from *Salmonella abortus equi* (originally sold as Lipexal®, Dorsey Co.) has been used clinically in healthy volunteers. Granulocytosis is the most sensitive parameter induced by endotoxin, followed in ascending order by fever, plasma cortisol and growth hormone response[20]. Man and rabbit are approximately equisensitive to the effects of endotoxin, an increase in body temperature being caused with 0.001–0.005 µg/kg i.v. of *Salmonella abortus equi*[20,21]. In the present study, prostaglandin E production increased 35–53 min after endotoxin, and this is in agreement with the finding that an increase in PGE is associated with the onset of fever (R. C. Skarnes, personal communication). In man, no clinical reaction is seen until 45–90 min postendotoxin and the side effects associated with increasing temperature are chills, rigor, headache, myalgia, anorexia, nausea and sometimes vomiting[21]. Many of these reactions are observed following administration of prostaglandins. Whether prostaglandins would be effective as post-coital contraceptive agents is not yet known. In Japan, it has been claimed that oral doses of 1–1.5 mg of PGE_2 per day administered from the 5th to the 10th day after ovulation had a contraceptive effect in 6 women during 50 cycles[22]. This effect seems to be more directed to prevention of implantation than interference with ovum transport. It may be that the shortlasting but unpleasant side effects of endotoxin preclude its use as a contraceptive although it is not clear that they are worse than the longer-lasting effects of large doses of estrogen. The biological activity of endotoxin appears to reside in the lipid portion of the molecule[23] and whether the toxic effects can be separated from the desirable contraceptive ones by judicious manipulation of the structure remains to be determined.

Table 1. Effect of endotoxin injected i. v. at 24 h after hCG on ovum transport in rabbits killed at 48 h after hCG

Treatment	No. of rabbits	No. of corpora lutea	Ova in oviducts No.	%	No. of ova in uterus or vagina
Control	13	158	127	80	5
Endotoxin 1 µg/kg	4	41	29	71	2
5 µg/kg	4	48	22	46*	1
10 µg/kg	6	56	7	13*	0
20 µg/kg	7	77	14	18*+	13
Endotoxin (20 µg/kg) plus indomethacin (10 mg/kg) i.m.	4	37	31	84*+	1

* Significantly different from control at $p < 0.001$ (χ^2 test with Yates' correction). + Significantly different at $p < 0.001$ (χ^2 test with Yates' correction).

Table 2. Concentrations of prostaglandins in uterine vein plasma of rabbits

Time from endotoxin (min)	Observed values (ng/ml) PGF n	PGF (mean ± SE)	PGE n	PGE (mean ± SE)
0	6	0.93 ± 0.24	6	0.36 ± 0.12
35– 53	4	1.17 ± 0.36	5	1.35 ± 0.35
85– 98	4	9.22 ± 5.56	5	2.22 ± 0.31
115–117	2	14.69 ± 6.44	2	1.65 ± 0.06
157–182	3	11.56 ± 2.39	4	2.46 ± 0.59
223	1	9.23	1	3.51
500–578	3	5.80 ± 1.64	3	3.22 ± 0.89

* Deceased 6 December, 1977.

1 Thanks are due to Elizabeth Menchaca, Larry Coons and Lenore Weinberg for technical assistance, and to Merck, Sharp, and Dohme for the gift of indomethacin. This work was supported by Rockefeller Foundation, NIH (HD-09339) and WHO (S76119). G. V. is a Rockefeller Foundation Postdoctoral Fellow in Reproductive Biology. Reprint requests to M. J. K. Harper.

2 M. C. Chang, in: Ovum Transport and Fertility Regulation, p. 473. Ed. M. J. K. Harper, C. J. Pauerstein, C. E. Adams, E. M. Coutinho, H. B. Croxatto and D. M. Paton. Scriptor, Copenhagen 1976.
3 M. C. Chang and D. M. Hunt, Nature *236*, 120 (1972).
4 M. C. Chang, D. M. Hunt and C. Polge, Adv. Biosci. *9*, 805 (1973).
5 J. V. Ellinger and K. T. Kirton, Biol. Reprod. *11*, 93 (1974).
6 M. C. Chang, S. K. Saksena and D. M. Hunt, Prostaglandins *5*, 341 (1974).
7 W. K. Phillipp-Dormston and R. Siegert, Naturwissenschaften *61*, 134 (1974).
8 F. K. Anderson, T. J. Tsagaris, W. Jubicz and H. Kuida, Am. J. Physiol. *228*, 1479 (1975).
9 A. G. Herman and J. R. Vane, Eur. J. Pharmac. *39*, 79 (1976).
10 R. C. Skarnes and M. J. K. Harper, Prostaglandins *1*, 191 (1972).
11 R. J. Flower and J. R. Vane, in: Prostaglandin Synthetase Inhibitors, p. 9. Ed. H. J. Robinson and J. R. Vane. Raven Press, New York 1974.
12 B. Caldwell, S. Burstein, L. Brock and L. Speroff, J. clin. Endocr. Metab. *33*, 171 (1971).
13 W. A. Stylos, S. Burstein, B. Rivetz, P. Gunsalus and R. C. Skarnes, IntraSci. Chem. Rep. *6*, 67 (1972).
14 W. A. Stylos, L. Howard, E. Ritzi and R. Skarnes, Prostaglandins *6*, 1 (1974).
15 M. C. Chang and M. J. K. Harper, Endocrinology *78*, 860 (1966).
16 C. J. Pauerstein, A. Sabry and B. J. Hodgson, Fert. Steril. *27*, 1308 (1976).
17 I. Aref and E. S. E. Hafez, in: Ovum Transport and Fertility Regulation, p. 320. Ed. M. J. K. Harper, C. J. Pauerstein, C. E. Adams, E. M. Coutinho, H. B. Croxatto and D. M. Paton. Scriptor, Copenhagen 1976.
18 L. K. Kuchera, Contraception *10*, 47 (1974).
19 A. A. Haspels, Contraception *14*, 375 (1976).
20 S. M. Wolff, J. infect. Dis. *128*, Suppl. S 259 (1973).
21 S. E. Greisman and R. B. Hornick, J. infect. Dis. *128*, Suppl. S 265 (1973).
22 T. Ninagawa, M. Ohta, T. Hiroshima, Y. Tomita, K. Ito, N. Imoto and R. Matsukawa, in: Obstetrical and Gynaecological Uses of Prostaglandins, p. 55. Ed. S. M. M. Karim. Medical and Technical Publ., Lancaster UK, 1976.
23 O. Luderitz, C. Galanos, V. Lehmann, M. Nurminen, E. T. Rietschel, G. Rosenfelder, M. Simon and O. Westphal, J. infect. Dis. *128*, Suppl. S 17 (1973).