Results and discussion. In the tied off colon of control rats, water net flux was directed from lumen to blood. Net absorption of water was 0.84 ± 0.08 ml/h. 1 µg/ml bisacodyl reduced net absorption of water ($p < 0.05$) and 10 µg/ml bisacodyl and 100 µg/ml phenolphthalein reversed net absorption into net secretion ($p < 0.01$). The latter was also found with mannitol solution (4%). PGE_1 (2 µg/ml) applied into the lumen of the tied off colon, totally abolished net absorption of water and caused a net secretion of 0.06 ± 0.12 ml/h (not shown in the figures). Pretreatment with indomethacin (4 mg/kg day, s.c., starting 2 days prior to the experiment) reduced the effect of 1 µg/ml and 10 µg/ml bisacodyl ($p < 0.01$ and $p < 0.05$) and of phenolphthalein ($p < 0.01$); it did not influence net absorption of water in control animals. The effects of mannitol, an osmotic laxative, was not changed by pretreatment with indomethacin. This indicates that diphenolic laxatives might exert their action via PG-release, since indomethacin inhibits PG-biosynthesis[9–11] (figure 1).

In the perfused colon of control rats, water net flux was also directed from lumen to blood. Net absorption was 1.46–0.20 ml/h, that means 1.74 times higher than in the tied off colon. Accordingly, the effects of all laxatives tested were weaker in the perfused colon. To get comparable effects, higher concentrations had to be used in the perfused colon than in the tied off colon. No explanation for this discrepancy can be offered at present. Bisacodyl (50 µg/ml) and mannitol both reduced net water absorption markedly ($p < 0.01$) and phenolphthalein (500 µg/ml) caused net water secretion ($p < 0.01$) (figure 2). The concentration of phenolphthalein was 10fold the concentration of bisacodyl, according to their therapeutic potency[12]. During control periods, the total amount of PGE, released into the perfusate, was 250 ± 97 pg/h. During perfusion with bisacodyl (50 µg/ml), PGE-release increased about 3fold ($p < 0.01$) and with phenolphthalein (500 µg/ml) about 4.5fold ($p < 0.01$). Mannitol (4%) did not increase PGE release (figure 2).

It is suggested that diphenolic laxatives exert their action via stimulation of PGE-biosynthesis in the colon.

9 S. H. Ferreira, S. Moncada and J. R. Vane, Nature New Biol. *231*, 237 (1971).
10 J. B. Smith and A. L. Willis, Nature New Biol. *231*, 235 (1971).
11 J. R. Vane, Nature New Biol. *231*, 232 (1971).
12 F. H. L. Van Os, Pharmacology *14*, 18 (1976).

The effects of hydrocortisone and glycyrrhizine on the enzyme releases of arylsulfatase and hyaluronidase from lysosomes of liver

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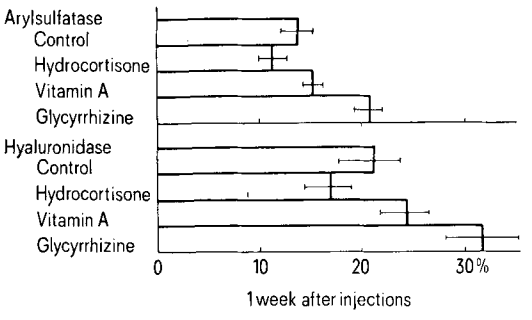
Summary. Hydrocortisone and glycyrrhizine act as both stabilizers and labilizers of the lysosomes of liver. The effect of both agents on the lysosomes is changeable according to the duration of their administration.

Lysosomes, which contain many hydrolytic enzymes, take part in the destruction and the restoration of tissue in inflammation. These lysosomal enzymes are released from the lysosome in the inflammation of the tissues. Some labilizing and stabilizing agents of lysosomes are well known[2–4].

Material and methods. Male albino rats of the Wistar strains, weighing approximately 200 g at the beginning of the experiement were used. Chronic hepatic damage was induced by i.m. injections, twice a week for 8 weeks, of 3ml of carbon tetrachloride per kg b. wt. Experimental animals were divided into 4 groups. Each group consisted of 7 rats. In the vitamin A group, vitamin A was administered by i.m. injection, of 10,000 IU/100 g b.wt, every other day for 2 weeks after the discontinuance of carbon tetrachloride. In the steroid hormone group, hydrocortisone was injected i.m. (dose 0.5 mg/100 g b.wt), every other day for 2 weeks after the discontinuance of carbon tetrachloride. A solution of 0.2% glycyrrhizine was administered by i.m. injection of 0.03 ml/100 b. wt every other day for 2 weeks after the discontinuance of carbon tetrachloride. The control animals received an appropriate volume of physiological saline after the discontinuance of carbon tetrachloride. The animals were killed by decapitation at 1 and 2 weeks after the administration of vitamin A, hydrocortisone and glycyrrhizine. The livers were taken out after being bled, and 8% liver homogenates were prepared. A supernatant of 20,000 × g in 8% homogenate was prepared. Each fraction of homogenate and supernatant was treated with ultrasound. Optimum liberation of particle-bound enzyme without loss of activity was obtained at 60 kW for 120 sec (Kubota 200 M). The enzyme assay of the total arylsulfatase activity was perfomed by the method of Worwood[4]. Hyaluronidase activity with hyaluronic acid as the substrate was measured by the method of Hutterer[5].

Results. The figure shows the pattern of the percentage of the release of the lysosomal enzymes at 1 week after dosing. The result of the administration of hydrocortisone



Percentage release of lysosomal enzymes at 1 week after dosing. Percentage release: Specific activity of supernatant specific activity of homogenate × 100.

1 Research Institute for Tuberculosis, Leprosy and Cancer, Tohoku University, Sendai, Japan.

2 G. Weissmann and L. Thomas, Recent Prog. Horm. Res. *20*, 215 (1964).
3 J. T. Dingle, Biochem. J. *79*, 509 (1961).
4 L. Merkow, M. Pardo, S. M. Epstein, E. Verney and H. Sidransky, Science *160*, 79 (1968).
5 M. Worwood, K. Dogson and F. A. Rose, Biochem. J. *134*, 183 (1973).

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]

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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-