proliferation of the smooth endoplasmic reticulum[5]. The present study suggests that the decrease of intestinal G-6-Pase activity will occur along with the normal decrease of the amount of endoplasmic reticulum[5] instead of being the result of a control exerted by thyroxine, cortisone or insulin. However, even though these hormones are unable to induce the decrease of G-6-Pase, they are still able to provoke regional increases of activity as shown in the table. The physiological significance of these small but still significant increases is still unknown. This is the first report concerning the study of a possible implication of hormones in the regulation of the post-natal decrease of G-6-Pase activity in the small intestine. Recently, it has been shown that thyroxine (1 µg/g b.wt) administrated to developing rats decreases hepatic G-6-Pase activity[16]. On the other hand, it has been reported that hydrocortisone and insulin have no effect on this activity in pre- and postnatal liver[17]. In conclusion, even though the G-6-Pase activity decreases when the brush border hydrolytic functions increase, the hormones known to influence or even control the postnatal development of brush border membrane-bound enzymes are not involved in the regulation of the post-natal decrease of the endoplasmic reticulum membrane-bound G-6-Pase activity of the intestinal epithelial cells.

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Biliary excretion of sulfobromophthalein in isolated perfused livers from normal and spironolactone-treated rats[1]

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Summary. Bile flow and biliary excretion of sulfobromophthalein (BSP) was examined in isolated perfused livers from normal and spironolactone(SP)-treated rats. BSP biliary excretion contributed to the bile production in both groups. Moreover SP increased BSP biliary excretion but transfer of dye from plasma into liver was not affected.

Sulfobromophthalein (BSP) has been widely used in the evaluation of liver function and its handling by the isolated liver was considered useful to test the applicability of the model in the study of drug metabolism[3]. Transfer of BSP from the plasma to the bile has been shown to consist of 4 separate steps: a) translocation from plasma into the liver by a carrier-mediated process[4,5], b) binding to a cytoplasmic protein denominated ligandin[6], c) conjugation with glutathione[7], d) excretion into bile mainly in the conjugated form[8]. Bile flow is also an important parameter in determining the rate of excretion of BSP into the bile[9], but decreased bile flow after administration of large doses of BSP has been reported[10]. Biliary excretion of BSP may be increased by microsomal enzyme inducers. The effect of these compounds might be due to their ability to increase bile flow[11], whereas ligandin seemed not to be involved in the phenomenon[12]. Spironolactone (SP) has been shown to be an important inducer of bilirubin metabolism in the rat liver[13,14], and to increase bile flow in this species by enhancing the bile salt-independent fraction of canalicular bile[15]. This steroid accelerates the elimination of BSP in living rats not only by increasing bile flow but also by enhancing enzymic BSP-glutathione conjugation[16]. Since BSP, to a certain extent, enters all tissues of the body[17], it was of interest to avoid the potential problems of extrahepatic dye distribution. Therefore, in this study we examined bile flow and biliary excretion of BSP in isolated livers obtained from normal and SP-treated rats, using the model applied to single dose incorporation[18].

Materials and methods. Adult male Wistar rats weighing 300–350 g were used as donors of livers. A group of rats was injected with SP dissolved in propylene glycol at a daily dose of 240 µmoles/kg (100 mg/kg) i.p. for 3 consecutive days. Control rats were injected with 1 ml of propylene glycol. The rats were allowed free access to water and saline solution during treatment, and were maintained ad libitum on a standard laboratory pellet diet. The perfusion medium consisted of heparinized rat blood mixed in a solution of Krebs-Ringer-bicarbonate buffer (pH 7.4, total volume 110 ml) with a hematocrit value of 11%. Albumin concentration in the supernatant was determined by the Bromocresol Green method[19], and averaged 4.8 ± 0.2 mg/ml. The preparation was essentially that described by Brauer et al.[20] with some modifications[3]. A constant pressure of 14 cm of water was maintained at the portal vein level. Flow rate through the liver (Q) determined by a direct measurement[20] 2 ± 2 ml/min. After 30 min of equilibration, a single dose of BSP of 16.2 ± 0.4 µmoles (13.5 ± 0.2 mg) dissolved in 6 ml 0.9% NaCl solution, was introduced into the reservoir. The ratio BSP to albumin was well below the binding capacity of albumin estimated for BSP[4]. Perfusate samples were obtained from the prehepatic and posthepatic circuits, at 5 min after the injection, and then every 2–3 min for 20–25 min. Bile was collected at 10-min intervals for 60 min. The volume of bile was estimated by gravimetry. The concentrations of BSP in samples of perfusate (after centrifugation), and bile (diluted 1:100 with distilled water) were determined by colorimetry after alkalination with

The handling of BSP by the isolated perfused rat liver

	Controls (n=5)	Spironolactone pretreatment (n=3)	p-Value
Body wt of liver donors (g)	314 ± 4	315 ±8	NS
(Liver wt/body wt) 100	2.8 ± 0.2	3.8 ±0.1	<0.001
Rate of perfusion (Q, ml/min/g of liver)	6.23± 0.57	5.56±0.51	NS
Mean bile flow (µl/10 min/g of liver)	9.8 ± 0.2	19.3 ±1.2	<0.001
BSP retained at 5 min after the injection of dye (% of dose)	31.0 ±10.7	30.3 ±6.1	NS
Mean extraction ratio (E)	0.20± 0.06	0.25±0.03	NS
Mean hepatic clearance (Ch, ml/min/g of liver)	1.60± 0.18	1.47±0.32	NS
BSP excreted 40 min after the injection of dye (% of dose)	36.5 ± 7.0	68.4 ±6.0	<0.02
Bile BSP concentration (nmoles/ml)*	35.0 ± 4.3	23.6 ±1.4	<0.05

Data are mean values ± SEM. NS, not significant. * Concentrations measured on the peak of BSP biliary excretion rate.

0.1 N NaOH by means of a Spectrophotometer set at 580 nm[3,21]. It was previously observed that decay of BSP in the pre- and posthepatic circuits showed a parallelism[3]. The areas under the inflow (A_i) and outflow (A_o) perfusate concentration time curves were calculated[3,18] and the following parameters were estimated: mean extration ratio ($E=A_i-A_o/A_i$), mean hepatic clearance (Ch, ml/min = QE), percent dose retained in the perfusate at 5 min after the injection, rate of BSP biliary excretion, bile BSP concentration, and percent dose excreted during the first 40 min after administration[3,18]. Livers were weighed at the end of the perfusion, and the results expressed per g of liver. The ratio liver wt/body wt was also calculated. The relative amounts of free and conjugated BSP were estimated in bile samples, after concentration to a small volume[22], separation of the spots by ascending paper chromatography[23], development of the spots by spraying with 5% (w/v) KOH[24], and densitometry.

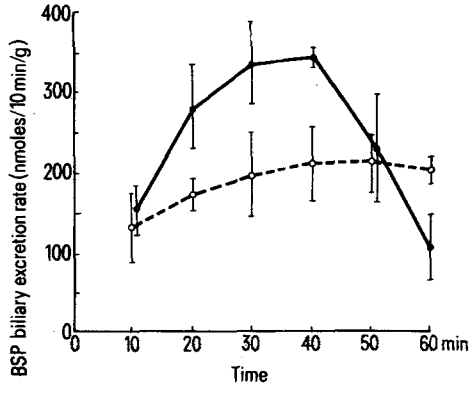

Figure 1. Sulfobromophthalein excretion rate by the isolated perfused rat liver. Results are means ± SEM for 5 control livers (○ – – – ○) and for 3 spironolactone-treated livers (●———●).

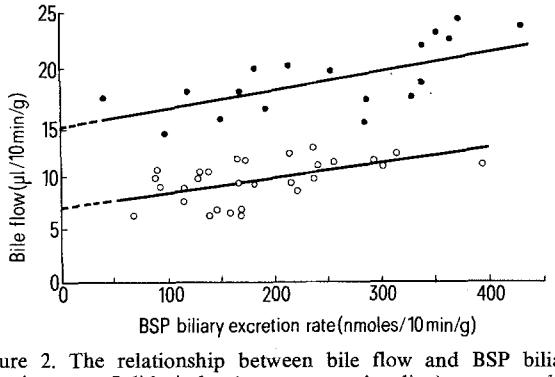

Figure 2. The relationship between bile flow and BSP biliary excretion rate. Solid circles (upper regression line) correspond to spironolactone-treated livers and open circles to control livers. For other details see text.

Results and discussion. SP pretreatment resulted in a significant increase of the ratio liver wt/body wt, bile flow and percent dose of BSP excreted after 40 min. BSP retained at 5 min, E, and Ch, showed no differences between groups whereas BSP concentration in bile of SP-treated livers was decreased. The results are presented in the table. The rate of biliary excretion of BSP in control livers reached a maximum 40 min after injection, whereas that of SP-treated livers showed a peak between 30 and 40 min after injection, with a higher rate of BSP biliary excretion (fig. 1). When bile flow values were plotted against BSP biliary excretion rate a direct relationship was obtained in both experimental groups (controls: $y=7.031+0.015$ x, $r=0.58$, $n=30$, $p<0.001$; SP: $y=14.767+0.018$ x, $r=0.60$, $n=18$, $p<0.01$). Extrapolation of the regression line to zero BSP excretion gave a positive intercept giving an estimate of the rate of bile flow in the absence of BSP (fig. 2). Results of paper chromatography showed that the percent of conjugated BSP present in bile was similar in both groups (controls: 71.0±0.3%; SP: 72.0±3.2%).

The results described in this paper by using an isolated rat liver preparation were in agreement with data obtained in living rats concerning the effect of SP in enhancing bile flow and biliary excretion of BSP at the expense of conjugated BSP[16]. Since bile flow was increased by SP, it was not possible to eliminate the possibility that the increase in BSP excretion rate produced by the steroid might be at least in part a consequence of increasing bile flow as suggested[9,11,16]. Controversely, the rate of BSP biliary excretion apparently contributed to the bile production independently of treatment. Therefore, diminished concentration of BSP in bile samples of SP-treated livers, may be due to an additional bile water excreted due to the increase of bile acid-dependent or independent fraction of bile[15], or both[25]. The present studies also demonstrated that removal of dye in each circulation and in the first 5 min after injection, was not influenced by SP. This agreed with preliminary observations (unpublished) where the initial disappearance rate of BSP from plasma in living rats, expressed as the first order rate constant K_1[26], was unchanged by SP (SP-treated rats 0.240±0.012, controls 0.270±0.013). Thus, we can assume that BSP transfer from plasma into liver is not affected by SP.

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Influence of oxygen concentration on development of *Drosophila melanogaster*[1]

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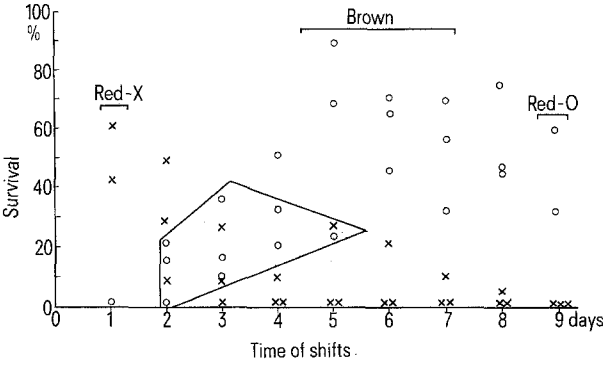
Summary. 1st, 2nd, and early 3rd instar *Drosophila* larvae are extremely sensitive to 100% O_2 or 75% O_2/25% N_2 (at atmospheric pressure) whereas eggs, late 3rd instar larvae, and pupae are relatively insensitive under our exposure conditions. Eclosing flies exposed to an O_2 enriched environment consistently possessed 2 eye abnormalities: dark eye color and altered eye shape.

Approximately 1.3 billion years ago[2] photosynthesis provided sufficient free oxygen (1% of its present value) for utilization of a superior strategy (aerobic metabolism) for the production of high energy phosphate bonds. Compared to anaerobic processes, metabolism with free O_2 confers a 20:1 efficiency advantage. However, all taxa appear to be sensitive to oxygen poisoning[3], and it is generally accepted that oxygen is potentially toxic at all concentrations[4]. For aerobes, mechanisms have evolved which protect tissue from oxygen-generated free radical damage at normal (air) concentrations. These mechanisms include catalase, glutathione peroxidase, superoxide dismutases, and DNA repair mechanisms[2,4]. Current literature supports a model which suggests that the inability of these mechanisms to manage oxygen-generated superoxide and hydroxyl radicals and hydrogen peroxide, causes the accumulation of undesirable or non-functional cellular substances associated with aging tissue[5,6].

The sensitivity of adult *Drosophila* to poisoning by oxygen has been extensively documented[3,7-9]. While the effects of short-term elevated O_2 exposure are largely reversible, long-term exposures apparently damage the nervous system[3,9]. In this paper we describe the influence of increased atmospheric oxygen (at normal atmospheric pressure) on development of 2 *Drosophila* strains, *vestigial wings*[10] (at position 67.0 on chromosome 2) and wild type (Urbana). The vestigial strain was selected because of its shortened life span[11] and reported decrease of superoxide dismutase activity in adults when compared to wild type[12].

Eggs were collected at 8 h intervals and placed 50-each in shell vials containing 5 cm^3 of standard cornmeal, molasses, sucrose, agar medium, Each vial was stoppered with a single layer of coarse-mesh nylon. Such vials were initially placed in a lucite chamber containing either a 20% O_2/80% N_2 gas mixture or a similar chamber containing 100% O_2. At 24-h intervals 1 (or 2) vial from each chamber was reciprocally shifted[13]. After 14 days from the onset of each experiment the percentage of surviving adults was determined. 3 replications of this procedure using a total of 5850 eggs provided the points (represented by symbols O = 20% O_2/80% N_2 → 100% O_2; X = 100% O_2 → 20% O_2/80% N_2) presented in the figure. A zone of overlap exists in days 2 through 5 thereby indicating that larvae are highly sensitive to 100% O_2 at 1st, 2nd, and early 3rd instars. Eggs, late 3rd-



Percentage survival (adult flies) of developing vestigial *D. melanogaster* exposed to 100% O_2 and transferred to a 20% O_2/80% N_2 gas mixture (X) and the reverse (O) at 24-h intervals at room temperature (24 ± 1 °C). See table for explanation of Brown, Red-X and Red-O terms. Symbols in the same column represent results from 3 replicate experiments conducted on a total of 5850 eggs.