12 mice were used for each dose level. The doses used are reported in the Table. Groups of animals were kept in separate cages for 72 h. Results were subjected to probit analysis. Medium lethal dose (LD_{50}) with the fiducial limits and potency ratios with the 95% confidence limits were obtained. Acceptable tests for parallelism and heterogeneity were always obtained before the potency ratios were determined.

Results. The NaCN induced deaths of the control mice and of the mice treated i.v. with sodium pyruvate, which occurred within less than 10 min, are shown in the Table. There was no evidence of late toxicity.

Sodium pyruvate treatment resulted in a marked increase in the amount of NaCN required to produce death. The values of LD_{50}, reported in the same Table, deviate significantly ($p<0.05$).

From these data it is evident that pyruvate treatment has an antagonist effect against acute cyanide lethality in mice.

The prompt regression of the symptomatology of cyanide intoxication, together with the markedly decreased incidence of lethality, indicate that there is a strict correlation between these effects of pyruvate in vivo and the release of the cyanide-blocked respiration already observed in isolated cells[5]. Moreover, even though additional pharmacological experimentation is required, the present data suggest the possibility of using pyruvate as an antidote to acute cyanide poisoning.

Riassunto. Sono stati studiati gli effetti del piruvato di sodio nella intossicazione acuta da cianuro nel topo. I risultati, statisticamente validi, dimostrano che il piruvato iniettato per via endovenosa riduce notevolmente l'effetto letale del cianuro di sodio somministrato per via endoperitoneale[6].

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Increased Bilirubin Conjugation in the Liver and Intestinal Mucosa of Phenobarbital Treated Rats

It has been shown that the intestinal mucosa is able to conjugate several substrates with glucuronic acid and that this capacity is parallel to that of liver[1]. This ability has also been proved in vitro for bilirubin[2,3]. In a previous work[4] we demonstrated the presence of conjugated bilirubin in the intestinal mucosa of normal and hepatectomized rats infused intravenously with unconjugated bilirubin.

Phenobarbital administered to patients with jaundice, due probably to bilirubin UDP-glucuronyl transferase deficiency, led to a reduction of plasma bilirubin levels[5]. Since phenobarbital increased the maximum biliary excretion of bilirubin (Tm) in heterozygous Gunn rats with a partial defect of bilirubin conjugation, enzyme induction by drug administration had to be admitted[6]. On the other hand, the level reduction of plasma unconjugated bilirubin in jaundice by phenobarbital treatment was considered strong evidence of bilirubin conjugation deficiency[7].

In this investigation we found that phenobarbital increased the content of conjugated bilirubin in the intestinal mucosa of Wistar and heterozygous Gunn rats infused with unconjugated bilirubin intravenously. This effect was also observed after total hepatectomy.

Methods. 24 Wistar and 20 heterozygous Gunn rats of both sexes weighing from 260 to 400 g were used. 12 Wistar and 9 heterozygous Gunn rats were used as untreated controls. The remainders were injected daily with phenobarbital (100 mg/kg dissolved in 1 ml of 0.9% NaCl) i.p. during 3 days. Unconjugated bilirubin was infused i.v. as described[4] and the bile collected for Tm calculation[8]. Blood, liver, as well as the mucosa and content of the small intestine, were obtained as previously reported[4]. Total bilirubin was determined in serum and bile samples[9] and in liver, mucosa and intestinal content homogenates[4,10]. Diazotized samples were concentrated to a small volume[11] and chromatographed on paper[12]. Conjugated bilirubin was calculated from total bilirubin concentration and the proportion of azopigment B on the chromatograms determined by densitometry (Densicord 542 A, Photovolt, USA).

In another set of experiments, 4 Wistar and 5 heterozygous Gunn rats (2 rats of each group received phenobarbital) were subjected to total hepatectomy[4] and then injected i.v. with unconjugated-^{14}C bilirubin (200,000 to 350,000 dpm, specific activity 2,400 to 4,200 dpm/μg) mixed with unradioactive pigment (2 mg/100 g body wt.). The animals were sacrificed 30 min after the injection. Crystalline radioactive bilirubin was obtained from the bile of rats[13] that were injected with ^{14}C-ALA (δ-aminolevulinic acid-4-^{14}C hydrochloride, CEA, France)[14]. Mucosa and intestinal content homogenates were diazotized and chromatographed as described. Azopigments B were eluted from chromatograms, pooled separately, concentrated to a small volume and submitted to two-dimensional chromatography. After the first development, the sheet was removed, dried and azopigments B were hydrolized on the paper[2] by using bacterial β-glucuronidase (Sigma Che.Co.) (1% solution in distilled water alcalinized with 0.1 *N* NaOH at pH 6.2). After incubation at 37 °C the

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sheets were rechromatographed at right angles to the first chromatography. Densitometry and scanning of the spots (4π Scanner, Tracerlab, USA) were carried out before the hydrolysis and after the second chromatography.

Results. Determination of bilirubin in serum and liver homogenates showed similar variations in the 4 groups (Figure 1). Liver weight expressed as g of wet tissue per 100 g of body wt. was 3.2 (SE ± 0.14) and 3.1 (SE ± 0.21) in Wistar and heterozygous Gunn rats respectively. There was no significant difference after phenobarbital treatment. Tm values as shown in Figure 2 were diminished in heterozygous Gunn rats as compared to the Wistar group ($P<0.001$) and a significant increase was observed in the former after phenobarbital treatment ($P<0.01$). Total bilirubin in the intestinal mucosa showed similar values in untreated rats but after receiving phenobarbital increased significantly in both groups ($P<0.05$ in Wistar group and $P<0.02$ in heterozygous Gunn rats). The differences were due to the increase of conjugated bilirubin concentration ($P<0.01$ and $P<0.02$, respectively). In heterozygous Gunn rats treated with phenobarbital the percentage of mucosa conjugated bilirubin increased to twice the values observed in their controls (Figure 3A). Total bilirubin in the intestinal content did not show differences among the 4 groups. However conjugated bilirubin increased significantly in heterozygous Gunn rats after phenobarbital treatment ($P<0.05$) (Figure 3B). In 2 homozygous (jaundiced) Gunn rats pretreated with phenobarbital and infused with unconjugated bilirubin, no conjugated pigment could be detected in the intestinal mucosa.

A direct relationship existed between Tm values (due mainly to bile conjugated bilirubin) and the percentages of conjugated bilirubin determined in the intestinal mucosa of treated and untreated rats ($r = 0.56$, $P<0.001$).

The percentage of conjugated bilirubin in the intestinal mucosa of hepatectomized heterozygous Gunn rats was also increased after phenobarbital treatment. Results of 2 dimensional chromatography showed that azopigments B from mucosa and intestinal content of hepatectomized rats (treated or not with phenobarbital) could be partially hydrolyzed with β-glucuronidase indicating that at least 43 to 48% was derived from bilirubin glucuronide. Before the hydrolysis only one peak of radioactivity was present in azopigment B area and a second peak was seen after the hydrolysis coincident with azopigment A spot.

Discussion. The experiments demonstrated the ability of the rat intestinal mucosa for bilirubin conjugation with glucuronic acid. Phenobarbital administered to rats exhibiting a partial deficiency of bilirubin glucuronoconjugation capacity may increase this latter in the liver as well as in the intestinal mucosa. It was not rejected that an increase of bilirubin uptake by tissue might be involved in the process, as reported for liver[15]. Recently 2 hepatic

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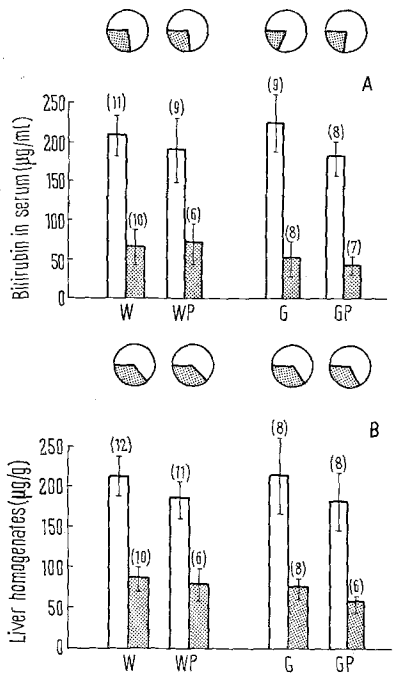

Fig. 1. Distribution of bilirubin in serum (A) and liver homogenates (B). W, Wistar rats; G, heterozygous Gunn rats; P, phenobarbital treatment. □, total bilirubin; ▒, conjugated bilirubin. The circles indicate the percentage of conjugated bilirubin. Between parenthesis, number of rats. —, Standard error of mean.

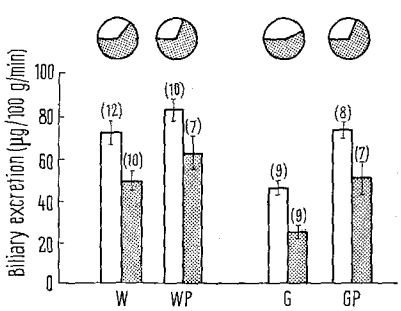

Fig. 2. Maximum biliary excretion of bilirubin (Tm). Explanatory legend is similar to Figure 1.

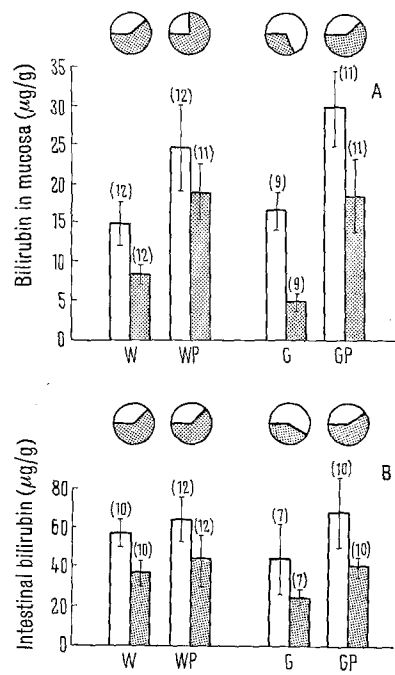

Fig. 3. Distribution of bilirubin in mucosa (A) and intestinal content (B). Explanatory legend is similar to Figure 1.

cytoplasmic protein fractions, Y and Z, which bind organic anions (such as bilirubin) have been isolated[16]. Whereas protein Y is present exclusively in the liver[16] and increases after phenobarbital administration[17], the small intestinal mucosa only contains fraction Z[16]. Failure of phenobarbital in increasing conjugated bilirubin concentration in the intestinal mucosa of homozygous Gunn rats is similar to that described for the liver[6]. This fact and the correlation between Tm values and percentages of mucosa conjugated bilirubin seem to confirm that glucuronide synthesis by the intestinal mucosa is parallel to the liver capacity[1,4]. We detected some conjugated radioactive pigment in the gut lumen of hepatectomized rats after the injection of labelled unconjugated bilirubin. However, the mechanism of transfer was not established[18,19].

Resumen. Se estudió el efecto del fenobarbital sobre la conjugación de la bilirrubina en ratas Wistar y Gunn heterocigotas sometidas a la infusión continua de bilirrubina no conjugada. Los resultados obtenidos permiten suponer que el fenobarbital es capaz de estimular la conjugación de la bilirrubina tanto en el hígado como en la mucosa intestinal de ratas con deficiencia parcial de glucuronil transferasa.

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Ultrastructure of the Parathyroid and 'C' Cells of the Thyroid in Experimental Rachitis in the Rat

In young rats submitted to a diet[1] characterized by an increased calcium/phosphorus ratio and a lack of Vitamin D, a decalcification of the formed bone and an absence of calcification in the growing bone was produced. These animals showed some histopathological alterations of the gastric mucosa with a significant increase of HCl. Macroscopic ulcerations in large quantities were also observed[2].

Supposing that the diet directly influences the regulatory mechanisms of calcium and phosphorus, we studied the morphology of the thyroid glands ('C' cells) and the parathyroid glands since serum calcium levels were the same in the treated rats as in the non-treated which were used as the control group[3].

Material and methods. Wistar rats, 25 to 30 days old, weighing 45 to 55 g and of aleatory sex were used and they were put on the diet immediately after weaning. Blood samples were taken by decapitation of treated and non-treated rats in basal conditions. The samples were allowed to coagulate at room temperature and were then centrifuged. The inorganic phosphate was determined by a photocolorimetric micromethod[3] utilizing a Beckman-DU photocolirimeter for its readings.

For the ultrastructural study, the parathyroids and thyroids were taken under anaesthesia with uretane moments before the decapitation. The fragments obtained were immediately fixated in glutaraldehyde (2 h) and osmium tetroxide (1 h), buffered by phosphate buffer to pH 7,2 at a temperature of 4 °C. Dehydrated with acetone, included in vestopal, cut with an ultramicrotome LKB, contrasted with uranil and lead compounds and observed with a Phillips EM-200 electron microscope.

Results. The quantity of inorganic phosphate can be seen in the Table.

Parathyroids. The principal cells were characterized by a greater electronic density in the nucleus and cytoplasm, and by the emission of numerous microvillis on the surface which intercrossed with those of neighboring cells, leaving between them clear spaces (Figure 1). The number of mitochondria and of rugous endoplasmic reticulum were moderate in their development. The Golgi apparatus appeared very well developed (Figure 1). Around it were a few low-density granules surrounded by a nitid membrane. Abundance of free ribosomes and polysomes (Figure 1). The centrioles were remarkably developed so that even ciliated images were seen (Figure 1). Frequently myelinic bodies were observed in the cellular cytoplasm and in the interstitial spaces (Figure 1). The nuclei with evident nucleolus and dense chromatin, separated below double nuclear membrane, showed wide gashes. The control rats of the same age clearly showed the principal cells with their characteristic types – clear and dark – (Figure 2A).

Thyroids: Numerous calcitonin cells were observed with abundant secretory granules which were a size from 1200 to 2500 Å of a fine granular matrix, and surrounded by a very evident mebrane. The Golgi apparatus was well-developed and showed, at its border small, very dense granules. (Figure 2B)

Serum levels of inorganic phosphorus in normal (C) and rachitic rats (R)

Groups	P		Significance
C	(9)	4.00±0.2172[a]	NS
R	(7)	3.97±0.2516[a]	NS

P = mg/100 ml. [a] Mean±standard error. In parenthesis, number of determinations.

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