Effect of phosphate omission on the glucose-induced insulin (IRI) release

$H_2PO_4^-$ (mM)	Without basal glucose		With 2.7 mM basal glucose	
	1st phase (ng/5 min)	2nd phase (ng/25 min)	1st phase (ng/5 min)	2nd phase (ng/25 min)
3.6	27.2±4.7	84.3±8.9	45.5±5.3	277.3±49.2
0.0	7.6±1.4	27.4±6.8	18.1±9.2	56.2±21.3
Statistical analysis	$p<0.01$	$p<0.01$	NS	$p<0.01$

Number of experiments as indicated in the figure. NS, not statistically significant.

albumin (Armour), and 2% (w/v) dextran T-70 (Ibys). The experiments were performed both in the absence and in the presence of 2.7 mM glucose in the perfusate during the pre-stimulation period. The medium at 37 °C was continuously gassed with O_2 and CO_2 (95:5). After a 30-min pre-stimulation period, glucose (Merck) was infused through the pancreas using a side-arm perfusion pump at a rate calculated to reach a final concentration of 16.7 mM in the perfusate. The stimulation period lasted 30 min. Samples (2 ml) were collected into chilled tubes and frozen at −20 °C until assayed. Insulin (immunoreactive insulin, IRI) was measured as described[10]. All results are expressed as mean ± SEM and statistical analysis was performed using Student's t-test.

Results and discussion. Both in the presence as in the absence of phosphate in the medium, 16,7 mM glucose elicited a biphasic insulin secretion (figure). At 3.6 mM phosphate, the presence of 2.7 mM glucose in the perfusate during the pre-stimulation period, clearly potentiated the insulin secretion in response to 16.7 mM glucose. In the absence of phosphate, both 1st and 2nd phases of insulin release were significantly reduced (table).

Furthermore, in the absence of phosphate, the potentiation of the 16.7 mM glucose-induced insulin release by 2.7 mM basal glucose was severely impaired. These results are in contrast to previous reports which show that the absence of phosphate increased the arginine-induced insulin release when compared to controls with 1.2 mM phosphate[6]. Using the isolated islets from ob/ob mice, it has been reported that phosphate acted as an inhibitor of the glucose-induced insulin release[8]. The mechanism whereby phosphate influences insulin secretion remains to be elucidated. Several reports show that stimulation with insulin secretagogues may trigger a rapid, transient efflux of 32-P from pre-labelled pancreatic islets[1-5]. In conclusion, from the present investigation and from the data so far available in the literature, it is established that extracellular phosphate anions could be involved in the modulation of insulin release from the stimulated beta-cell.

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Measurement of urinary steroid production rates using stable-isotopes and GC-MS

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Summary. The urinary production rate of pregnenolone has been determined for a male subject using 7,7-d_2-pregnenolone as an isotopic tracer.

The recent general availability of deuterated steroids[2] in high isotopic purity offers the possibility of developing practical clinical procedures for the determination of urinary steroid production rates. These procedures should overcome most of the methodological and hazard drawbacks and also provide more precise estimates than those previously reported using radioactive tracers. The contention is supported by the following study in which the measurement of the urinary pregnenolone production rate is made using a stable-isotope steroid analogue.

A sterile solution of 7,7-d_2-pregnenolone[2] (0.8 mg ml^{-1}; propylene glycol: water, 3:1) was injected into the antecubital vein of a male subject (age 41 years) and urine collection instituted 2 days prior and 3 days post administration of the dose. Aliquots (10 ml) of the total urine collections were specifically hydrolysed (solvolysis or β-glucuronidase, ex coli) to release free steroids from the sulphate and glucuronide fractions respectively. Previous studies have shown that steroid recoveries by these procedures are >80%[3,4]. The required steroids 5-pregnene-3β,20α-diol (5PD) and 5β-pregnane-3α,20α-diol (PD) were further purified by TLC and derivativatized to form the corresponding pentafluoroacyl derivatives[5,6]. GC-MS employing selected ion monitoring[7] was then used to measure the enrichment factors ($ef = d_2(d_0+d_2)^{-1} \cdot 100$) for both 5-PD (M/z 446, 448) and PD (M/z 448, 450). The results are contained in the table.

No measurable quantity of urinary pregnenolone could be found in the samples and accordingly the enrichment factor was measured for 5-PD as this steroid has been indicated in previous studies[8,9] to satisfy the criterion as a 'unique' metabolite of pregnenolone.

From the results in the table it is apparent that there is a linear relationship between the dose and enrichment factor of 5-PD, suggesting no major alterations in clearance or metabolism at the higher doses. Total urinary steroid profiles[3] for the pre and post collections were almost identical again implying that no pharmacological affect was manifested because of the administered steroid.

The close agreement of the enrichment factors from the sulphate and glucuronide fractions of 5-PD (table) confirms previous findings[8] and suggests that the endogenous secretion of pregnenolone sulphate is low[10]. Expectedly, the enrichment factor for PD in most cases is considerably lower than that of 5-PD (table) since it is formed from other sources as well as via peripheral conversion of pregnenolone to progesterone[8]. Substitution in equation (1)[11] gives an average urinary pregnenolone production rate of 5.5 ± 1.3 mg $\cdot$ 24 h^{-1} which is in agreement with prior reports using radioactive isotopes[8].

Enrichment factors for 5-pregnene-3β,20α-diol (5-PD) and 5β-pregnane-3α,20α-diol (PD)

Steroid dose (mg)	Enrichment factors* (atom %) 2.0	3.6	6.0
5-PD			
Sulphate	7.9 ± 1.2**	21.0 ± 1.7	31.1 ± 2.1
Glucuronide	9.3 ± 1.0	19.4 ± 0.8	27.7 ± 1.4
PD			
Glucuronide	3.1 ± 0.5	14.2 ± 0.9	27.0 ± .18

* Mean ± SD based on ion ratio measurements (n = 8) corrected for background. ** Intra-assay CV = 5.0% (n = 4).

$$\text{Production rate} = \frac{a \cdot m}{ef \cdot t} - \frac{m}{t} \quad \ldots (1)$$

a = isotopic purity (%), m = mass (mg), t = days of collection

Extension of this work to more complicated systems in which the use of more than one labelled steroid is required[12] is presently under consideration.

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Oxygen consumption in the rat following neonatal thyrotoxicosis

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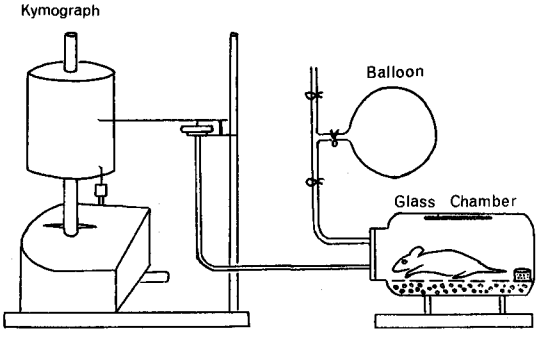
Summary. Adult rats made thyrotoxic with large doses of thyroxine during the neonatal period had a lower oxygen uptake as compared with neonatal controls and those of neonatal calorically-deprivated ones.

Rats treated with large doses of thyroxine during the first few days of life (neonatal thyrotoxicosis) results in permanent dysfunction of the hypothalamus-hypophyseal-thyroid system. Adult animals with neonatal thyrotoxicosis exhibit a lower level of protein bound iodine and free thyroxine plasma concentration, smaller thyroid glands, and the pituitary thyrotropin content is diminished[1,2]. Since the hormone influences oxidative metabolism in tissues[3,4], changes in the activity of the gland will be reflected directly by changes in the amount of oxygen utilized. The present investigation is concerned with the effect of an excess thyroxine at birth on the adult oxygen consumption.

Materials and methods. Litters of an inbred colony of Sprague-Dawley rats were randomly divided into different groups, each containing males and females. One third of rats in each litter were injected s.c. with 28 µg of L-thyroxine in 0.05 ml of 0.1 N sodium hydroxide on day 1 and continuing through the 7th day of age. An additional third served as controls, received only the alkaline diluent. Since newborn rats given excess doses of thyroxine lose weight, the effects of neonatal thyrotoxicosis and of neonatal weight loss produced by restricting food intake were compared. For the first week one third of the rats in each litter were removed from their mother for about 8 h daily and were injected as described above with alkaline diluent. After weaning at 4 weeks of age, the animals were fed a laboratory diet and water ad libitum. At the age of 60 days, the oxygen consumption of rats was measured in a glass metabolic chamber (figure) in which carbon dioxide was absorbed by a layer of soda lime on the floor, thus creating a fall in pressure within the chamber as oxygen was consumed. Water vapour produced by the animals were absorbed by calcium chloride in a small container in the glass chamber. The drop of pressure in the chamber was recorded on a Kymograph. The time for the volume of gas in the chamber to fall 40 ml was the time taken for the rat



Apparatus for determination of oxygen consumption in the rat.