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uridine diphosphate glucuronyl transferase
uridine diphosphate xylosyl transferase

Xylose, Glucose, and Glucuronic Acid Conjugation of Bilirubin in the Newborn Rat

SERGIO L. VAISMAN, KWANG-SUN LEE, AND LAWRENCE M. GARTNER[25]

Division of Neonatology, Department of Pediatrics, The Rose F. Kennedy Center for Research in Mental Retardation and Human Development and the Liver Research Center, Albert Einstein College of Medicine, Bronx, New York, USA

Extract

The development and relative contribution of hepatic bilirubin conjugation with glucuronic acid, xylose, and glucose was studied *in vitro* in newborn rats 1–20 days old. In adult control rats, 75% of the conjugates formed were with glucuronic acid, whereas in 1-day-old newborns, only 50% of the conjugates were with glucuronic acid ($P < 0.02$) while xylose and glucose conjugates of bilirubin together were equal to that of glucuronic acid. By *day 4*, total conjugating capacity increased to adult levels and a mature pattern of distribution. In response to phenobarbital treatment, xylose and glucose conjugation increased 4 days earlier than glucuronide conjugation and maximal induction occurred 8 days sooner for nonglucuronide conjugation than for glucuronide.

Speculation

The pattern of enzyme maturation and relative activities of the various bilirubin conjugating systems in newborn rats cannot be extrapolated to those in the human, but this study suggests that a similar study in the human or subhuman primate might demonstrate that glucose and xylose conjugation compensate, in part, for deficient glucuronide conjugation.

In mammalian species bilirubin is conjugated primarily with glucuronic acid at the site of the hepatic endoplasmic reticulum (microsomes) to form the water-soluble form of bilirubin for excretion into bile. It has long been recognized that fetal and newborn liver of all species is deficient in capacity to form the glucuronide conjugate of bilirubin because of marked reduction in bilirubin glucuronyl transferase activity (2, 13, 20). Recent study in newborn monkeys has demonstrated that the inability of the newborn liver to form bilirubin conjugates is largely responsible for the early phase of physiologic jaundice (7, 8).

Other carbohydrate conjugates of bilirubin, in addition to glucuronides, are formed in all species studied thus far, as has been demonstrated *in vitro* (4, 5) and by chromatographic separation of the pigments in bile (4, 11). These studies have been limited to the adult age group and the formation of nonglucuronide conjugates by the fetus and newborn has never been examined. Consideration must be given to the possibility that nonglucuronide conjugates of

bilirubin may compensate for deficient glucuronide conjugation during neonatal life.

We have previously demonstrated that 5-day-old Dornjou rats treated with phenobarbital during the first 4 days of life failed to demonstrate the expected induction of bilirubin glucuronide formation *in vitro* (17). The present study in the Sprague-Dawley strain of rats similarly has demonstrated no response to phenobarbital treatment during the first 4 days of life. This raised a second question as to whether drug induction of nonglucuronide conjugates by the newborn rat was either similarly unresponsive or, possibly, more responsive.

This study was undertaken to evaluate the development and relative contribution of three different bilirubin conjugating systems in the newborn rat, both in the native state and after phenobarbital stimulation. The three enzymes studied were uridine diphosphate glucuronyl transferase (UDPGT), uridine diphosphate xylosyl transferase (UDPXT), and uridine diphosphate glucosyl transferase (UDPGST) for the conjugation of bilirubin with glucuronic acid, xylose, and glucose, respectively.

MATERIALS AND METHODS

Sprague-Dawley pregnant rats were obtained from Marland Breeding Farms, Wayne, N. J. They were kept in individual breeding cages and allowed to deliver spontaneously. The newborn rats were studied on *days 1*, *4*, *8*, *12*, *16*, and *20* of life. Phenobarbital from Amend Drug & Chemical Co., Inc. was prepared in 0.1 N NaOH and pH adjusted to 8.4 with 12 N HCl and used for only 48 hr. Phenobarbital (PB) was administered subcutaneously in doses of 15 mg/kg once every 24 hr for the 4 days immediately preceding study for the newborns killed on *days 8*, *12*, *16*, and *20*. In the group studied on *day 1*, PB was administered to the pregnant mother from *day 18* to *21* of pregnancy in doses of 100 mg/kg·day, subcutaneously. In the group killed on *day 4*, PB was administered to the pregnant mother on the 21st day of pregnancy (100 mg/kg) and to the newborn from *day 1* to *3* (15 mg/kg). The control group received the same volume of 0.1 N NaOH, pH 8.4. Adult female rats weighing 260–290 g were treated either with PB at a dose of 100 mg/kg·day for 3 days or received an equal volume of 0.1 N NaOH, pH 8.4.

All animals were weighed daily and immediately before death. Control and PB-treated rats were exsanguinated under ether anesthesia 24 hr after the last injection. The livers were immediately removed, washed in ice-cold 0.154 M KCl, and homogenized. For the determinations on *days 1* and *4* several livers were pooled (3–13 livers for one determination). The mean for each day represents 6–12 determinations. Assays of bilirubin UDPGT, UDPXT, and UDPGST were immediately performed using whole liver homogenates, digitonin activated, with optimal concentrations of the following, obtained from Sigma Chemical Co., St. Louis, Mo.: UDP glucuronic acid (UDPGA, 6.19×10^{-2} M), UDP xylose (UDPX, 7.46×10^{-2} M), and UDP glucose (UDPG, 6.56×10^{-2} M). A micromodification of the previously described method was utilized so that all volumes were reduced to one-fifth of that previously used (6).

UDPGT, UDPXT, and UDPGST activities were expressed as micrograms of bilirubin conjugated per liver per 40 min after correction for blank (no UDPGA, UDPX, or UDPG). Total liver protein concentration was measured according to the method of Lowry (14). Student's *t*-test was used for determination of statistically significant differences between groups at a significance level of $P < 0.05$ for the two-tailed test (15).

RESULTS

UDPGT, UDPXT, AND UDPGST ACTIVITIES

In the control group, total conjugating capacity *in vitro*, calculated by summation of the three enzyme activities, increased from a low level (20% of adult normal) at birth to reach adult activity by *day 4* (Fig. 1). Total enzyme activity exceeded the adult level by about 30% on *days 12*, *16*, and *20* (Fig. 1). PB administration did not produce any increase in activity in animals

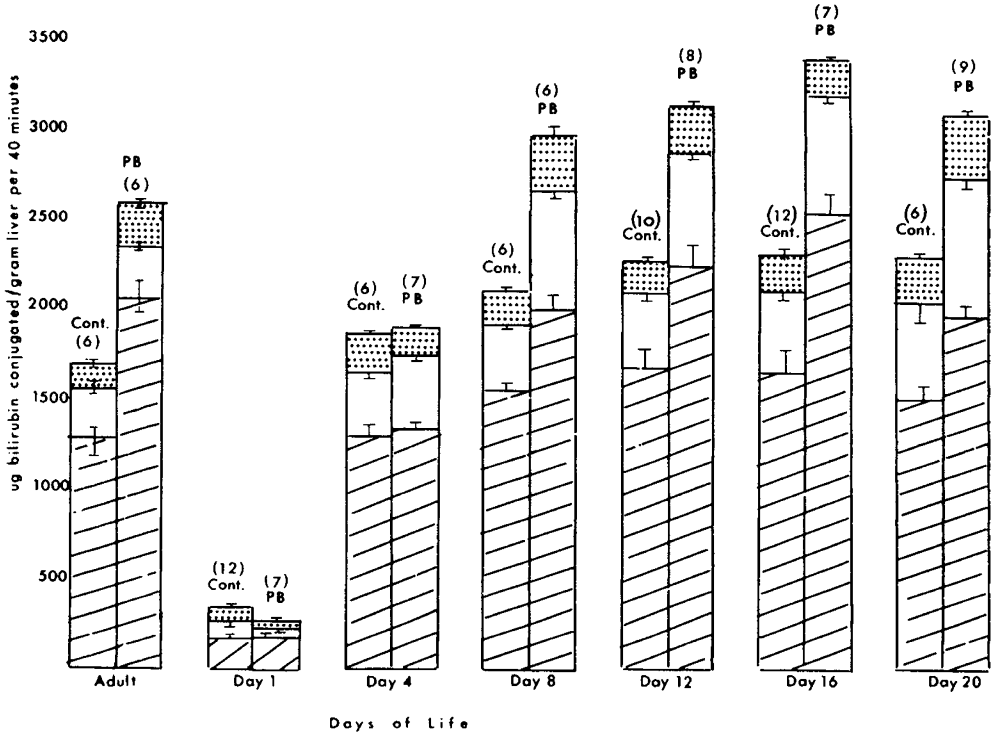

Fig. 1. Uridine diphosphate glucuronyl transferase (*hatched block*), uridine diphosphate xylose transferase (*open block*), and uridine diphosphate glucosyl transferase (*dotted block*) activities in newborn and adult rats with and without phenobarbital treatment. Activity is expressed as micrograms of bilirubin conjugated per g liver per 40 min (mean ± SE). Numbers in parentheses indicate the number of determinations in each group. *PB:* phenobarbital-treated group.

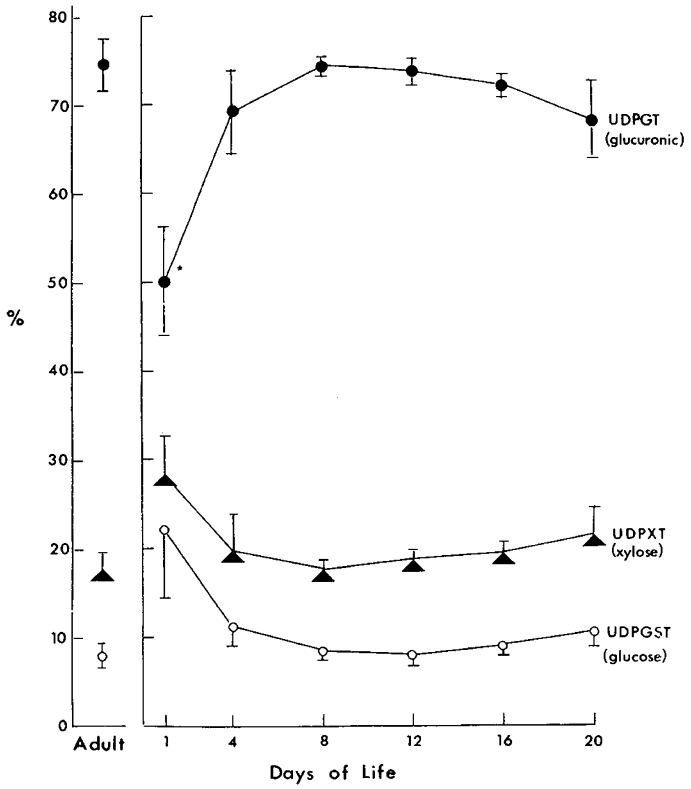

Fig. 2. Analysis of the contribution of uridine diphosphate glucuronyl transferase (*UDPGT*), uridine diphosphate xylosyl transferase (*UDPXT*), and uridine diphosphate glucosyl transferase (*UDPGST*) in adult and newborn untreated rats. Results are expressed as the mean percentage of distribution of each group ± SE. *: significantly different from adult distribution. Number of determinations in each group: adult, 6; 1 day, 12; 4 days, 6; 8 days, 6; 12 days, 10; 16 days, 12; 20 days, 6.

1 and 4 days old but on *days 8*, *12*, *16*, and *20*, significant increases of from 26 to 32% were seen ($P < 0.01$).

The analysis of the contribution of each of the three enzymes in the control animals is presented in Figure 2. UDPGT (glucuronic acid) activity in adult rats without PB stimulation accounted for 74.5% of total conjugating activity, whereas UDPXT (xylose) accounted for 17.6% and UDPGST (glucose) 7.9%. In the first day of life UDPGT activity accounted for only 50% of total activity, a significant reduction as compared with the adult ($P < 0.02$). UDPXT increased to 28% and UDPGST to 22% of total activity. These increases were not significant, however.

By *day 4*, the distribution was returning toward that of the adult and by *day 8* and thereafter was not significantly different from that of the adult.

The percentage of increase in conjugation with each of the three substrates after PB administration is illustrated in Figure 3. As noted previously for total conjugating activity, PB did not significantly increase the formation of any of three conjugates alone on *days 1* and *4* in the newborns. On *day 8*, UDPXT and UDPGST activities increased by 80% and 88%, respectively, in response to PB administration as compared with controls of the same age. This significant increase on *day 8* ($P < 0.01$) contrasts with insignificant increase of glucuronide formation ($P > 0.10$). By *day 12*, the degree of UDPXT and UDPGST enhancement after PB as compared with controls declined, although the degree of enhancement remained significant, whereas UDPGT enhancement became significant ($P < 0.01$). Peak UDPGT activity occurred on *day 16*, 8 days later than the peak enhancement for UDPXT and UDPGST.

LIVER WEIGHT

In the control group relative liver weight was greatest on *day 1*, decreased progressively to *day 8*, and increased after this time to reach adult size by *day 16* (Table 1). The PB-treated group had a similar pattern of progression in relative liver size, but liver weights on *days 4*, *8*, *16*, and *20* were significantly greater than controls.

LIVER PROTEIN CONCENTRATION AND TOTAL LIVER PROTEIN

In the control group liver protein concentration rose progressively from *day 1* to a maximum on *day 16* (Table 1). PB administration did not produce a significant change in liver protein concentration on any day of study, but total hepatic protein relative to total body weight was increased significantly on *days 8*, *16*, *20*, and in the adult rat.

If enzyme activities are expressed relative to body weight (micrograms of bilirubin conjugated per 100 g body weight per 40 min) or relative to liver protein concentration (micrograms of bilirubin conjugated per mg protein per 40 min), the pattern and degree of response to PB administration is the same as when expressed per liver mass.

DISCUSSION

In the untreated newborn rat we have found a rapid increase in total bilirubin conjugating capacity *in vitro* from *days 1* to *4*, as would be expected from previous reports. The previously reported patterns of maturation and activity of UDPGT (the only bilirubin

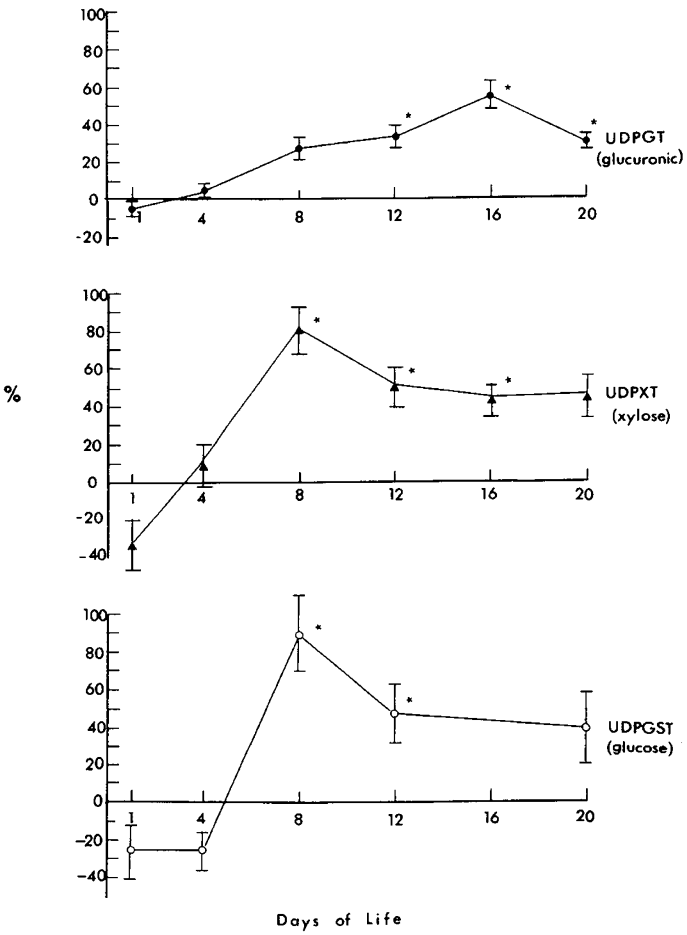

Fig. 3. Percentage of increase in uridine diphosphate glucuronyl transferase (*UDPGT*), uridine diphosphate xylosyl transferase (*UDPXT*), and uridine diphosphate glucosyl transferase (*UDPGST*) activities after phenobarbital treatment in newborn rats as compared with controls of the same age. Results are expressed as the mean ± SE. *: significant increase in activity. Number of determinations in each group: 1 day, 7; 4 days, 7; 8 days, 6; 12 days, 8; 16 days, 7; 20 days, 9.

Table 1. *Liver weights, liver protein concentrations, and total liver protein in adult and newborn rats with and without prior phenobarbital (PB) administration*[1]

Age, days	Liver weight, g/100 g body wt		Liver protein, mg/g liver		Total liver protein mg/100 g body wt	
	Control	PB	Control	PB	Control	PB
1	4.98 ± 0.19	4.71 ± 0.09	135 ± 4	135 ± 3	666 ± 22	636 ± 18
4	3.44 ± 0.19[2]	4.20 ± 0.06[2]	139 ± 7	138 ± 6	482 ± 43	582 ± 29
8	3.24 ± 0.05[2]	3.92 ± 0.09[2]	166 ± 5	152 ± 5	539 ± 16[2]	596 ± 14[2]
12	3.33 ± 0.03	3.39 ± 0.04	171 ± 6	188 ± 7	567 ± 19	637 ± 29
16	3.63 ± 0.05[2]	3.89 ± 0.08[2]	186 ± 8	207 ± 4	675 ± 29[2]	804 ± 22[2]
20	3.91 ± 0.03[2]	4.53 ± .0.10[2]	171 ± 5	177 ± 5	670 ± 20[2]	804 ± 34[2]
Adult	3.59 ± 0.10	3.78 ± 0.19	201 ± 4	212 ± 3	720 ± 22[2]	801 ± 33[2]

[1] All values are mean ± SE. Number of determinations in each group (control/phenobarbital): *day 1*, 12/7; *day 4*, 6/7; *day 8*, 6/6; *day 12*, 10/8; *day 16*, 12/7; *day 20*, 6/9; adult, 19/12.

[2] Significantly different, $P < 0.05$.

conjugating enzyme previously studied), relative to the adult, vary widely from one study to the next (1, 3, 10, 13). In general, however, all previous studies demonstrated maximal activity peaks at between 10 and 30 days with peak activities greater than those of adult controls. In the present study maximal total conjugating activity was achieved on *days 12* to *16* and exceeded adult mean activity by 30%. Peak activity for UDPGT alone was found on *day 12* and declined to adult levels by *day 20*.

During the first day of life the conjugation of bilirubin with glucuronic acid accounted for only half of the total capacity. The non-glucuronide conjugates contribute a significant portion of the capacity of the day-old newborn liver to form the water soluble conjugates of bilirubin. As glucuronide conjugation matures to its maximal activity, the non-glucuronide conjugates (glucose and xylose) become relatively less important. All three enzymes decline significantly by the 20th day. The explanation for this pattern of maturation is lacking.

The significance of the contribution of the non-glucuronide conjugates during the early days of life is supported by the fact that both xylose and glucose conjugation responds earlier than glucuronide conjugation to phenobarbital treatment. Maximal induction, as percentage of enhancement over control, was found on *day 8* for both glucose and xylose and on *day 16* for glucuronic acid. The greater contribution in the first days of life of xylosyl and glucosyl transferase may, in part, compensate for deficiency of glucuronyl transferase, helping to minimize bilirubin retention.

The failure of 4 days of PB administration to mother and newborn to increase UDPGT, UDPXT, or UDPGST activity on *days 1* and *4* contrasts with the only previous study in rats of bilirubin UDPGT activity after PB treatment, but this study used a different strain of rats and a quite different assay for measurement of UDPGT (10). Increased bilirubin UDPGT activity in response to PB has also been demonstrated in newborn mice (3) and monkeys (8). The failure to induce the enzyme during the first 4 days of life may relate to differences in species, strain, dosage, metabolism of PB, and enzyme assay techniques. Although metabolites of phenobarbital were not determined in the present study, fetal and newborn phenobarbital concentrations in rats were found to be identical with maternal hepatic concentrations (19). In the present assay system, whole homogenates were used. It is possible that similar studies performed with microsomal preparations might reveal significant increases in enzyme activity. If this were the case, it would suggest the presence of inhibitors of enzyme activity in the whole homogenate preparation. Evidence for the presence of inhibitors of glucuronyl transferase is yet to be presented. By the 8th day of life increased bilirubin conjugation was clearly demonstrable in the present study.

The mechanism of control of the observed differences in age-related distribution of the three enzymes is unknown, but several speculations may be considered. (*1*) Each of the three enzymes may be different and independent, increasing enzyme synthesis in the postpartum period at unrelated rates and responding to drug induction as separate proteins. (*2*) The low activity of each of the enzymes may be determined, in part or in whole, by the presence of an inhibitor(s), with each enzyme having a different susceptibility to the same or different inhibitor(s). (*3*) The three enzymes may be structurally related but dependent for their activity on the presence of three separate unidentified cofactors and with each cofactor becoming available at a different time postnatally.

The lipid composition of the liver microsomal membranes has been demonstrated to be an important determinant of the activity of glucuronyl transferase (9, 21). The different contributions of the various bilirubin conjugating systems in the newborn and the adult could be due to differences in the phospholipids of the microsomal membranes. Different transferases may require different phospholipids. Changes in hepatic microsomal fatty acid synthesis during development in the rat may account for the earlier maturation of UDPXT and UDPGST than UDPGT.

The type of conjugates formed *in vitro* are usually found to be present in the bile of the species studied, but the relative distribution of each conjugate in bile need not correlate with enzyme activity (4). Thus, the functional significance of this enzymatic study can only be determined by chromatographic study of bile from newborns, both laboratory animals and humans.

The patterns of enzyme maturation and relative activities in the rat may not represent the human situation. Thus, non-glucuronide conjugation may be of little or no importance in the human newborn. On the other hand, it is possible that in the human, xylose and glucose conjugates may be of even greater importance during the neonatal period than in the rat, particularly after drug stimulation. PB has been demonstrated to be effective in decreasing serum bilirubin concentrations in the human newborn (16, 18). The mechanism of this response is not fully understood but could be mediated through an increase in bilirubin conjugates other than glucuronic acid. Further studies are clearly indicated to evaluate the role of different conjugating systems in the human newborn.

SUMMARY

The development and relative contribution of three different enzymes for hepatic bilirubin conjugation (UDPGT, UDPXT, and UDPGST) were studied in the newborn rat. Total conjugating capacity (summation of three enzyme activities) reached that of adult controls by the fourth day of life. In the first day of life, UDPGT activity accounted for only 50% of total activity, a significantly smaller proportion of total activity compared with 75% in the adult. By the eighth day and afterward, the distribution of enzyme activities were the same as in the adult.

Maternal phenobarbital administration did not enhance the

activity of any of the three enzymes during the first 4 days of life. By the eighth day, however, UDPXT and UDPGST activities increased 80 and 88% as compared with controls, respectively. UDPGT demonstrated no enhancement on *day 8* in response to phenobarbital. By the 12th day, enhancement of UDPGT became significant, reaching maximal increase on *day 16*.

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25. Requests for reprints should be addressed to: L. M. Gartner, M.D., Department of Pediatrics, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, N. Y. 10461 (USA).

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Angiotensin II
Bartter's syndrome
plasma renin activity
renin-aldosterone axis
sodium
urinary excretion

Effect of Sodium Restriction and Angiotensin II Infusion in Bartter's Syndrome

L. SANN,[30] M. DAVID, P. RICHARD, D. FLORET, J. SASSARD, C. A. BIZOLLON, AND R. FRANCOIS

Hôpital E. Herriot and Hôpital Debrousse, Lyon, France

Extract

Five patients with Bartter's syndrome were investigated. Sodium restriction (<10 mEq/day for at least 5 days) showed a renal sodium wastage in only two patients (*I* and *II*) in spite of increased aldosterone secretion rate (from 151–427 to 680–842 μg/day). The effect of angiotensin II (A II) 80 ng/kg/min for 30–180 min, on plasma renin activity (PRA), plasma aldosterone, and urinary sodium excretion was compared with the effect of a previous infusion of 5% dextrose given at the same rate, 0.5 ml/min for 1 hr. A II infusion resulted in increased plasma aldosterone levels: from 236–330 pg/ml to 800–881 pg/ml in 30 min. This increase was also observed in *patient II* (from 139 to 600 pg/ml). PRA was decreased by A II infusion (from 1,142–2,462 to 121–1,625 ng/liter/min). In *patient IV*, this decrease in PRA was also observed when he was on a salt-restricted diet (from 1,934 to 370 ng/liter/min); but the minimal PRA was still higher (370 ng/liter/min) than with a normal diet (121 ng/liter/min). In no case could normal PRA level be obtained. A II infusion induced an increase in urinary sodium excretion only in the two patients with renal sodium wastage (from 80–90 to 265–230 μEq/min in 30 min). Urinary sodium excretion decreased in the other patients from (37.5–213 to 4.30–46 μEq/min) and fractional sodium excretion was reduced in *patient V* (from 0.56% to 0.45% at 30 min and to 0.29% at 120 min). No signifi-