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THE EFFECTS OF *p*-CHLOROPHENOXYISOBUTYRATE ON THE TURNOVER RATE AND DISTRIBUTION OF THYROID HORMONE IN THE RAT*

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Abstract—A single dose of *p*-chlorophenoxyisobutyrate (CPIB) caused an apparent displacement of ^{131}I-labeled thyroxine (T_4) from the plasma into the liver and it also increased the rate of T_4 disappearance from the plasma, liver, kidney, heart and skeletal muscle of the rat. Since T_4 disappeared at the same rate from all tissues, tissue T_4 must have been in equilibrium with plasma T_4. The effects of CPIB disappeared after 30–40 hr, and T_4 turnover rates regressed toward those seen in control animals. When CPIB was fed to animals for 2–3 wks, it caused the plasma PBI concentration to drop from 5·2 to 3·7 μg/100 ml. Although the absolute plasma "free T_4" concentration remained unchanged (2·5 mμg/100 ml), the relative amount of circulating hormone present as "free T_4" increased about 40 per cent when CPIB was given. The displaced T_4 was concentrated by the liver and to a lesser extent by the kidney, causing these organs to become "hyperthyroid" as evidenced by a large increase in the mitochondrial α-glycerolphosphate dehydrogenase activity. At the same time, the rest of the organism remained "euthyroid" as evidenced by a lack of change in metabolic rate and thyroid function.

THE ADMINISTRATION of *p*-chlorophenoxyisobutyrate (CPIB) to the rat produces a marked increase in liver mitochondrial α-glycerolphosphate dehydrogenase (GPD) or α-glycerol-3-phosphate: cytochrome C reductase (EC 1.1.2.1.) activity,[1, 2] but very little increase in the GPD activity of other tissues.[2] GPD activity in most tissues (excluding brain, lung and spleen) is under the control of the thyroid hormone,[3] and it is unique to observe a selective increase in GPD activity in only one of the tissues that responds to thyroxine (T_4). The drug itself is not thyromimetic, and some form of endogenous or exogenous thyroid hormone must be present to obtain the CPIB-induced increase in liver GPD.[2] Moreover, Osorio *et al.*[4] found that the liver concentrates T_4 when CPIB is fed, which may account for the preferential liver GPD response to CPIB. Since CPIB is bound to the same serum proteins as that occupied by T_4, it has been suggested that any T_4 displaced by CPIB might be taken up specifically by the liver.[5] However, little drop in serum PBI concentration has been observed in patients receiving CPIB,[6] and Chang *et al.*[7] found that, although high concentrations of CPIB displaced some T_4 from prealbumin binding sites, CPIB

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did not displace T_4 from the TBG fraction in human plasma. CPIB had even less effect on T_4 binding by rat serum proteins. It can also be argued that any T_4 immediately displaced from plasma proteins would be insufficient to sustain an elevated liver GPD concentration for 6 weeks.[2] Therefore, the CPIB-induced increase in liver GPD activity is mediated in some way through the action of the thyroid hormone, but the exact mechanism has not been determined.

The present investigation was undertaken to determine the effects of CPIB on the T_4-concentrating ability of a number of tissues, the plasma T_4 turnover rates, the plasma protein-bound iodine (PBI) and "free T_4" concentrations and the uptake of ^{131}I-iodide by the thyroid gland at 6 and 24 hr. Similar studies were sometimes carried out with salicylate and 2,4-dichlorophenoxyacetic acid (2,4-D) in order to compare their effects on T_4 metabolism with that of CPIB.

METHODS

Groups of either six or eight weanling male rats (Holtzman strain) were fed a purified sucrose–casein–corn oil diet[2] with or without added supplements of various drugs. The drugs were incorporated into the diet at the expense of the carbohydrate. Instead of the regular Phillips and Hart salt mixture, a comparable salt mixture containing less iodine (5 mg iodide as potassium iodide/100 g salts) was used so that plasma PBI determinations could be made.* Stable and ^{131}I-labeled T_4 solutions for s.c. injection† were prepared by dissolving T_4 in 0·9% saline adjusted to a pH of 8·5–9·0. A single subcutaneous injection of 0·2 μc of ^{131}I-iodide was given for the thyroid ^{131}I-uptake measurement,† and the thyroid glands (still attached to a small piece of trachea) were removed from animals sacrificed at 6 and 24 hr after the injection. All radioactive specimens were counted in a gamma-sensitive scintillation well counter to an accuracy of $\pm$ 2 per cent and were corrected for physical decay.

The animals, except those used in the thyroid^{131}I-uptake measurements, were sacrificed by decapitation and blood specimens were collected. Appropriate tissue samples were also collected and all measurements were made immediately on fresh tissue. The GPD assay procedure and the metabolic rate determination have been described previously.[3]

EXPERIMENTAL

Effect of CPIB on ^{131}I-labeled T_4 (T_4) uptake and turnover by various tissues*

In the first experiment, two groups of rats were fed either the basal purified diet (BD) or basal diet plus 0·3% CPIB (BD + CPIB) for 2 weeks. A single subcutaneous dose of 1 μc of T_4* in a total of 1 μg T_4 was then administered to each animal, and groups of six animals each were sacrificed at the time intervals shown in Table 1. One ml of serum and approximately 1-g samples of liver, kidney and skeletal muscle from each animal were assayed for their radioactive content. Serum values were calculated as per cent of the administered dose per milliliter and tissue radioactivity concentrations were calculated as per cent of the dose per gram of tissue (Table 1). Ratios of serum radioactivity to tissue radioactivity were calculated and further

* Whole serum or plasma PBI and "free T_4" concentrations were measured by Bio-Science Laboratories, Van Nuys, Calif.

† ^{131}I- labeled L-T_4 and iodide were obtained from Abbott Laboratories. The ^{131}I-T_4 had an average specific activity of 43 mc/mg and the iodide was carrier-free.

comparisons were made between the ratios obtained for CPIB-treated animals and control animals (Table 1). Animals on the CPIB diet had faster serum T_4* disappearance rates ($T_{\frac{1}{2}} = 15$ hr) than control animals ($T_{\frac{1}{2}} = 22$ hr). Furthermore, semi-logarithmic plots (using the data shown in Table 1) of kidney, liver and skeletal

TABLE 1. EFFECT OF FEEDING 0·3% CPIB IN THE DIET FOR 2 WEEKS ON THE UPTAKE AND TURNOVER OF RADIOACTIVITY IN DIFFERENT RAT TISSUES*

Diet	Time (hr)	% Dose/ml serum†	% Dose/g tissue†		
			Liver	Kidney	Skel. mus.
Basal diet + 0·3% CPIB	2	0·96 ± 0·05	0·50 ± 0·04	0·51 ± 0·05	0·06 ± 0·01
	6	2·89 ± 0·19	1·22 ± 0·09	1·36 ± 0·11	0·20 ± 0·01
	12	2·33 ± 0·11	0·97 ± 0·05	1·09 ± 0·06	0·21 ± 0·01
	17	1·96 ± 0·19	0·81 ± 0·07	0·89 ± 0·08	0·18 ± 0·01
	24	1·35 ± 0·10	0·52 ± 0·03	0·57 ± 0·02	0·11 ± 0·00
	30	0·95 ± 0·04	0·47 ± 0·04	0·57 ± 0·06	0·08 ± 0·01
	48	0·54 ± 0·05	0·19 ± 0·02	0·25 ± 0·02	0·04 ± 0·01
	72	0·20 ± 0·02	0·08 ± 0·03	0·10 ± 0·03	0·03 ± 0·00
Basal diet	2	1·13 ± 0·04	0·43 ± 0·01	0·56 ± 0·04	0·06 ± 0·01
	6	2·15 ± 0·23	0·83 ± 0·08	1·03 ± 0·07	0·18 ± 0·01
	12	2·66 ± 0·17	1·03 ± 0·07	1·12 ± 0·07	0·20 ± 0·01
	17	2·36 ± 0·08	0·77 ± 0·04	1·00 ± 0·08	0·20 ± 0·00
	24	2·22 ± 0·15	0·66 ± 0·03	0·82 ± 0·03	0·16 ± 0·02
	30	1·46 ± 0·09	0·59 ± 0·03	0·64 ± 0·04	0·11 ± 0·00
	48	0·98 ± 0·06	0·28 ± 0·03	0·38 ± 0·01	0·07 ± 0·00
	72	0·45 ± 0·03	0·16 ± 0·02	0·24 ± 0·12	0·03 ± 0·01

Time (hr)	Serum/liver CPIB / Serum/liver control	Serum/kidney CPIB / Serum /kidney control	Serum/skel. mus.CPIB / Serum/skel. mus.control
2	0·75	0·96	0·88
6	0·91	1·01	1·21
12	0·93	0·90	0·84
17	0·80	1·00	0·92
24	0·71	0·81	0·83
30	0·88	1·15	0·96
48	0·87	0·90	1·39
72	0·63	1·14	0·72
Mean	0·81	0·98	0·97

* Each animal received a single s.c. injection of 1 μc ^{131}I-T_4 in 1 μg T_4 at zero time; 6 animals were used per group.

† Values are expressed as the arithmetic mean ± S.E.

muscle concentrations of radioactivity versus time showed that all three tissues cleared the radioactivity at approximately the same rate ($T_{\frac{1}{2}}$ values averaged 22 hr for control animals on basal diet and 16 hr for animals fed CPIB). Since these values coincide with those obtained from serum radioactivity curves, the radioactivity found in liver, kidney and skeletal muscle must be in equilibrium with the radioactivity found in serum. If ratios of serum to liver, kidney and skeletal muscle radioactivity for CPIB-fed animals are compared with those obtained for control animals (Table 1), it is apparent that the livers of CPIB-treated animals show a preferential uptake of radioactivity.

In the second experiment, the effect of a single dose or pulse of CPIB was observed on: the uptake of T_4 by several tissues and the T_4 disappearance rates in the same tissues and in serum. Preceding the administration of CPIB, 1 μc of carrier-free (less

than 0·03 μg T_4/injection) ^{131}I-labeled T_4 (T_4*) was injected s.c./100 g of body weight into rats weighing approximately 200 g. Ten hr later, one-half of the animals received a single s.c. injection of 50 mg of CPIB/100 g body weight. A time period of 10 hr between the dose of T_4 and CPIB was selected because it has been shown that a maximum blood level of T_4 is obtained approximately 10 hr after a single injection of radioactive T_4.[8] After the administration of CPIB to one-half of the animals, 6 control animals without CPIB and 6 animals given CPIB were sacrificed at each of the time intervals indicated in Figs. 1–3. Serum samples were assayed for total radioactivity and for their radioactive PBI concentration (PBI*). The latter values were obtained by precipitating and counting the protein precipitates prepared from 1 ml serum by the addition of 1 ml of 20 per cent trichloracetic acid (TCA). All precipitates were washed twice with 10% TCA before they were counted. There were no significant differences in the per cent of plasma radioactivity present as PBI* between groups, and in each case 88–92 per cent of the plasma radioactivity was present as PBI*. A single injection of CPIB caused a precipitous drop in serum PBI* levels (Fig. 1),

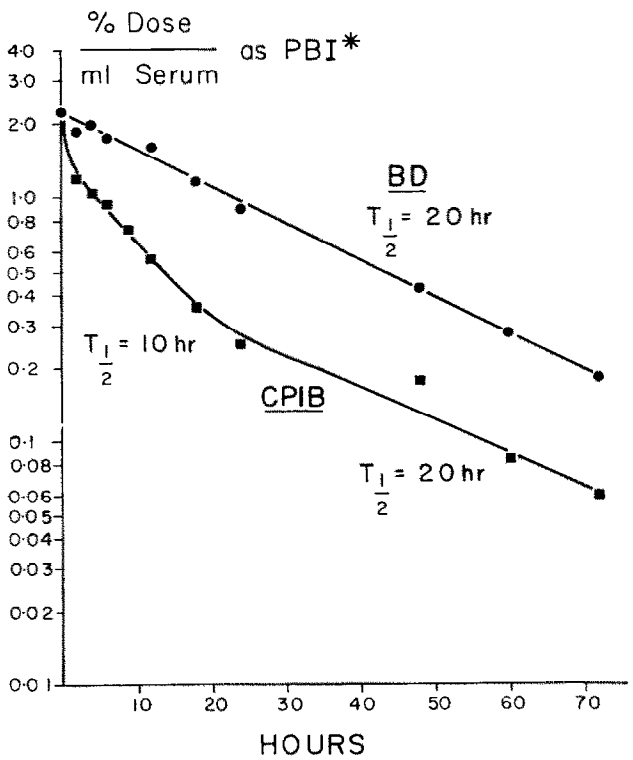

FIG. 1. Effect of a single dose or pulse of 50 mg CPIB/100 g body wt. on the serum PBI* concentration when serum T_4 was labeled beforehand by giving a single dose of 1 μc of carrier-free ^{131}I-T_4/100 g body wt. exactly 10 hr before the pulse of CPIB was administered. The time intervals shown are those at which the animals were killed after the pulse of CPIB.

and the T_4 disappearance rate in CPIB-injected animals was much faster ($T_{\frac{1}{2}}$ = 10 hr) than in control animals ($T_{\frac{1}{2}}$ = 20 hr). The effects of CPIB diminished after 30 hr and the disappearance rate of serum PBI* became nearly equivalent to that of the

control animals ($T_{\frac{1}{2}} = 20$ hr). At least a portion of the T_4 displaced from the serum proteins appeared to be concentrated in the liver and to a lesser degree in the kidney (Fig. 2), but heart and skeletal muscle showed no uptake of radioactivity over that of respective control tissues (Fig. 3).

It can be concluded from these experiments that CPIB causes a selective displacement of T_4 into the liver and to a lesser extent into the kidney. CPIB also effects a faster T_4 turnover rate as evidenced by the much shorter tissue half-time values obtained for CPIB-treated animals. The T_4 present in all tissues studied (including the liver, which concentrates T_4) must be in equilibrium with serum T_4 because T_4 disappearance rates in all tissues were the same and equal to that seen for serum PBI.

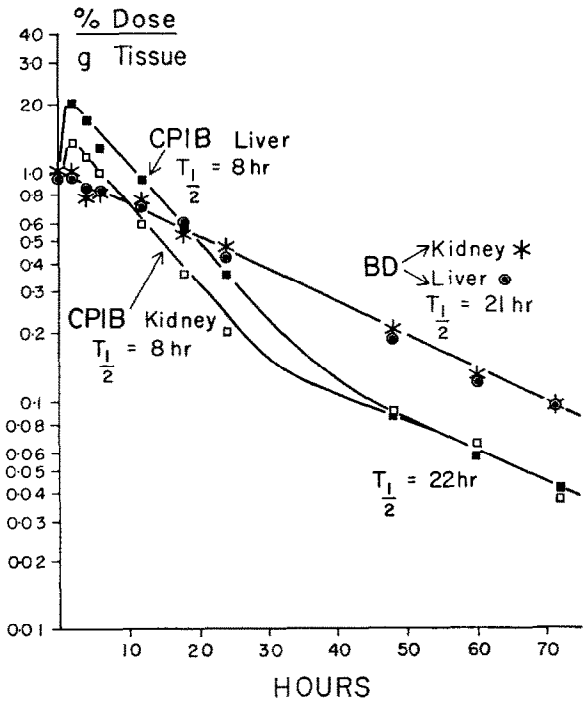

FIG. 2. Effect of a single dose or pulse of 50 mg CPIB/100 g body wt. on the uptake and turnover of ^{131}I activity in animals that had received a prior injection of ^{131}I-T_4. The experimental conditions and animals are the same as those employed in Fig. 1. The BD curves are for animals receiving the basal diet and those labeled CPIB are for animals given a single injection of 50 mg CPIB.

Effect of CPIB and other substances on liver GPD and serum PBI measurements after the elevation of serum PBI concentrations by the administration of large doses of T_4

Since CPIB appears to displace T_4 from serum protein under the above conditions, the following experiments were designed to study the effect of CPIB and other drugs on the turnover rates of experimentally elevated serum PBI concentrations. PBI values were elevated in two ways: 1) by giving 70 μg T_4 s.c./100 g body weight per day for 3 days to animals receiving 0·2% thiouracil (TU) in the basal diet, and 2) by giving 100 μg T_4/100 g body weight per day for 5 days to rats receiving the basal diet alone.

The first technique was used, since it has been shown that TU feeding produces abnormally high PBI values without much increase in GPD activity when T_4 is given along with TU.[8] Therefore, a large group of rats weighing 140–160 g were fed the basal diet plus 0·2% TU for 3 days, after which they received 70 μg T_4/100 g body weight for another 3 days while still on the TU diet. This caused the PBI to

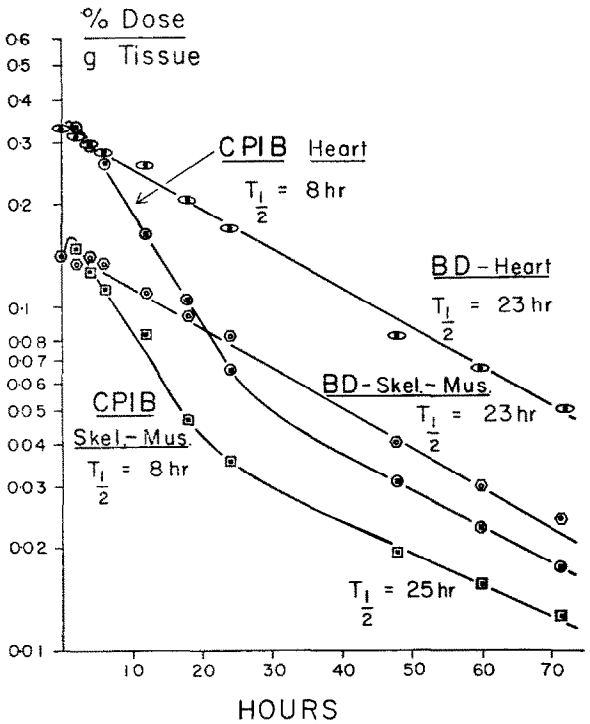

FIG. 3. Effect of a single dose or pulse of 50 mg of CPIB/100 g body wt. on the turnover of ^{131}I activity in animals previously given a single injection of ^{131}I-T_4 to label the endogenous pool of T_4. The experimental conditions and animals are the same as those employed in Figs. 1 and 2. BD curves are for animals receiving the basal diet and curves labeled CPIB are for animals given a single dose of 50 mg of CPIB. The abbreviation, Skel. Mus., represents skeletal muscle.

increase to 42 μg/100 ml of serum for the entire group of 24 rats. After these treatments, the animals were changed to the diets shown in Figs. 4 and 5, and T_4 injections and TU feeding were discontinued. At the time intervals shown, 6 animals from each group were sacrificed, and kidney and liver GPD activities and serum PBI concentrations were determined. All groups of animals, except those receiving 2,4-D, responded with an elevated liver GPD concentration. However, only CPIB sustained the GPD response, and previously elevated GPD levels in animals fed salicylate or 2,4-D fell at essentially the same rate as those seen in control animals receiving the basal diet. CPIB had little or no effect on the disappearance rate of serum PBI (Fig. 5) and the average $T_{\frac{1}{2}}$ for CPIB-fed animals was essentially the same (approximately 48 hr) as that seen for control animals. On the other hand, 2,4-D and salicylate caused a faster decrease in PBI concentration ($T_{\frac{1}{2}}$=approximately 32 hr) and the PBI values were always lower.

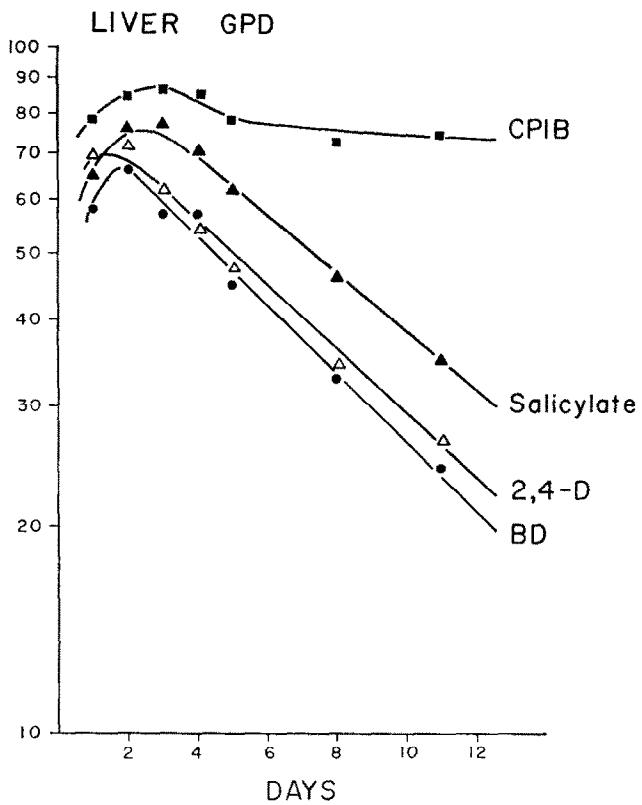

FIG. 4. Liver GPD response to CPIB and other drugs after the elevation of blood T_4 levels (PBI = 42 μg/100 ml for the entire group of 24 rats) by the prior administration of 70 μg T_4 s.c./100 g body wt./day for 3 days to rats receiving 0·2% thiouracil (TU) in the diet. After the plasma PBI was elevated, the animals were switched to the diets shown and 6 rats per group were sacrificed for the GPD assay at each of the time intervals shown. CPIB and salicylate were fed at a level of 0·3% (w/w) of the diet, and 2,4-D at a level of 0·15 per cent. Liver GPD values have the units of μl of O_2/10 min/150 mg tissues.

In the second experiment, 100 μg T_4 was given s.c./100 g body weight per day for 5 days to rats receiving the basal diet. At the end of this period, the animals were changed to the diets shown in Figs. 6 and 7. At the time intervals indicated, 6 animals from each group were sacrificed and liver GPD and serum PBI concentrations were determined. As in the previous experiment 0·3% CPIB in the diet stimulated liver GPD activity and these levels remained elevated over those seen in control animals on the basal diet (Fig. 6). The effects of feeding 10% bovine hemoglobin* (Hb) was tested, since it has been shown that Hb interferes with the absorption and enterohepatic recycling of T_4 in the rat by causing a greater fecal excretion of T_4 and hence a reduction in the serum T_4 concentration.[9, 10] In the present experiment, Hb caused a somewhat faster decrease in GPD activity in animals fed CPIB, but whereas the decline of liver GPD activity in animals receiving basal diet alone was reflected by a

* Crude dried bovine hemoglobin was obtained from the General Biochemicals Corp.

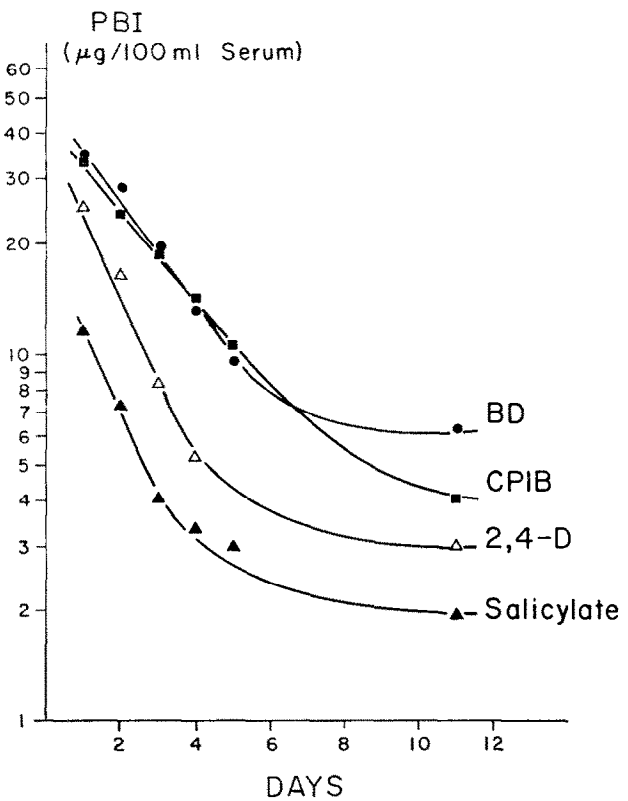

FIG. 5. PBI disappearance curves obtained from serum samples of the same animals used for the GPD analysis in Fig. 4. Each experimental point represents the average of 2 PBI values obtained from pooled serum samples from 3 rats each.

more rapid drop in serum PBI values, Hb did not enhance the already rapid fall-off in PBI concentrations of CPIB-fed animals (Fig. 7). The explanation for this difference is not known.

The effect of CPIB on previously elevated GPD and PBI values appears to depend, in part, upon the method used to elevate the values before the administration of CPIB. When TU was administered along with T_4, liver GPD values of CPIB-treated rats were elevated over those of controls; yet the PBI disappearance rate was essentially the same as that seen for controls on the basal diet. When PBI concentrations were elevated by giving T_4 alone, PBI concentrations of CPIB-fed rats fell more rapidly than those of controls. Thus, TU may have had a residual effect on the PBI concentration. Although interpretation of the 2,4-D and salicylate experiments may be similarly complicated by the prior administration of TU, both drugs caused liver GPD concentrations to remain below those of CPIB-fed animals and both drugs caused PBI values to be lower than those obtained for control and CPIB-fed animals.

Effect of feeding CPIB and other drugs on metabolic rate, liver and kidney GPD responses and on plasma PBI and "free T_4" concentrations

The same three drugs (CPIB, 2,4-D and salicylate) were fed to rats for 3 weeks in order to compare their effects on liver and kidney GPD concentrations and on plasma

PBI concentrations. Both 2,4-D and salicylate produced a marked reduction in plasma PBI values and they induced liver GPD responses equivalent to that produced by 0·15% CPIB (Table 2). A dietary level of 0·3 per cent 2,4-D was quite toxic. CPIB produced a smaller but significant reduction in the plasma PBI concentration, and it caused a larger percentage (about 40%) of the circulating T_4 concentration to

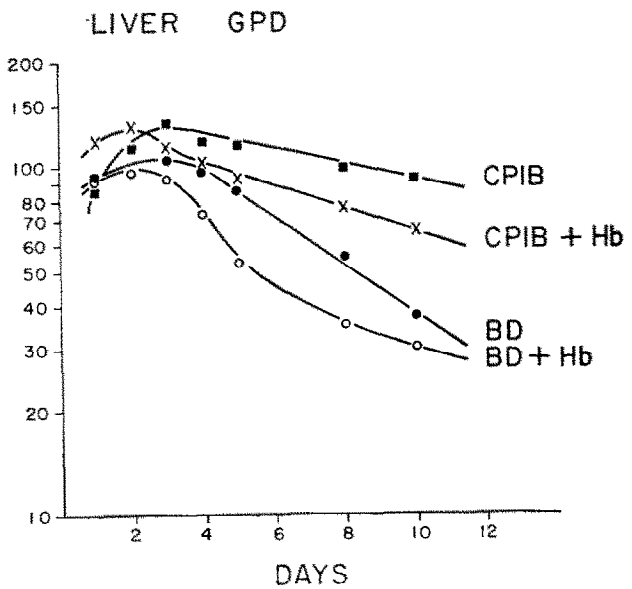

FIG. 6. Liver GPD responses to 0·3% CPIB and 10% hemoglobin (Hb) in the diet after the elevation of blood T_4 levels (PBI = 47 μg/100 ml plasma) by the prior administration of 100 μg T_4 s.c./100 g body wt./day for 5 days to rats receiving basal diet. After the 5-day period, the animals were switched to the diets shown and 6 rats per group were sacrificed at each of the time intervals. Liver GPD values have the units of μl O_2/10 min/150 mg fresh liver.

appear as "free T_4". The absolute "free T_4" concentration actually remained unchanged (2·5 mμg/100 ml plasma) from that found in control animals. In the case of all three drugs, some of the displaced plasma T_4 must have been picked up by the liver since a liver GPD response was observed without a corresponding change in kidney GPD activity.

Effect of CPIB and 2,4-D on the uptake of ^{131}I-iodide by the thyroid gland

In order to test the effect of CPIB on the iodine uptake by the thyroid gland, three groups of 16 rats each were fed the diets shown in Table 3 for 2 weeks, after which each animal received a single s.c. injection of 0·1 μc ^{131}I-iodide/100 g body weight. Eight animals from each group were killed at 6 and 24 hr after the injection. The thyroid glands were counted and the 6- and 24-hr uptake values were calculated as per cent of the dose of ^{131}I- administered. It is apparent from the data that CPIB did not appreciably affect the ^{131}I-uptake measurement, whereas 2,4-D produced a significant increase in ^{131}I-uptake.

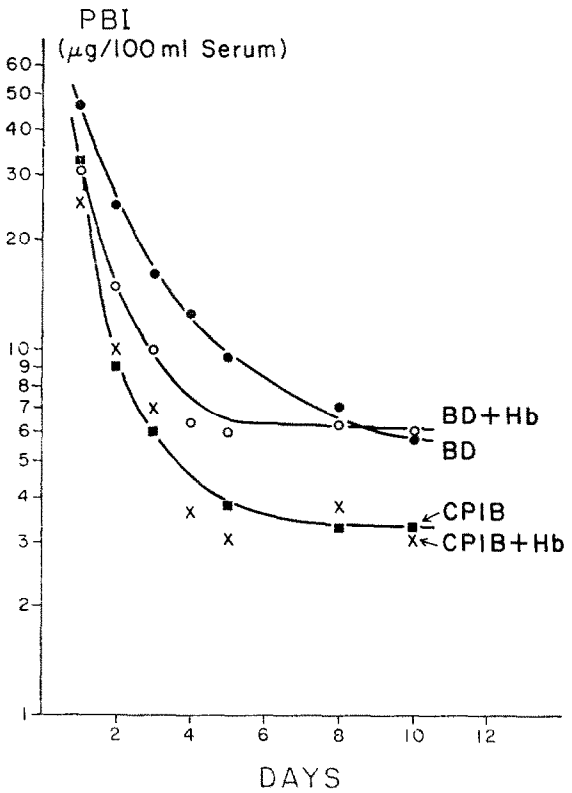

FIG. 7. PBI disappearance curves obtained from serum samples of the same animals used for the GPD analysis in Fig. 6. Each experimental point represents the average of 2 PBI values from pooled samples from 3 rats each.

TABLE 2. EFFECT OF FEEDING VARIOUS DRUGS FOR 3 WEEKS ON THE METABOLIC RATE, LIVER AND KIDNEY GPD RESPONSES AND ON PLASMA PBI AND "FREE T_4" CONCENTRATIONS

Group*	MR†	Liver GPD‡	Kidney GPD‡	Plasma PBI‡	Plasma "free T"‡
BD control	6·8 ± 0·7	28 ± 2·2	37 ± 2·8	5·2 ± 0·07	2·5 ± 0·19
BD + 0·3% CPIB	7·3 ± 1·1	132 ± 8·0	43 ± 2·4	3·7 ± 0·14	2·5 ± 0·07
BD + 0·15% CPIB		55 ± 3·2	40 ± 2·7		
BD + 0·15% 2,4-D		50 ± 4·1	39 ± 3·0	2·2§	
BD + 0·3% salicylate		45 ± 3·8	26 ± 2·7	2·7§	

* BD = the basal diet and 2,4-D = 2,4-dichlorophenoxyacetic acid. The values shown are the mean ± S.E. for 8 animals per group.

† Metabolic rate (MR) values have the units of liters of O_2/m^2 hr.

‡ Liver and kidney GPD concentrations are given as μl of O_2/10 min/150mg fresh tissue; PBI concentrations are in μg/100ml plasma; and plasma. "Free T_4" concenetrations are expressed as mμg/100 ml plasma.

§ Aliquots of plasma (1 ml) from each of the 8 animals were pooled for a single PBI determination.

DISCUSSION

Although CPIB administration brings about a large selective increase in liver GPD activity in the rat, this effect is achieved through the indirect action of the thyroid hormone. Neither thyroidectomized nor methimazole-treated rats show a liver GPD response to CPIB, but small replacement doses of thyroid hormone permit the

TABLE 3. EFFECT OF CPIB AND 2,4-D ON THE PER CENT DOSE OF ^{131}I-IODIDE TAKEN UP BY THE THYROID GLANDS*

Group	Per cent dose taken up	
	6 hr	24 hr
Basal diet (BD)	4·07 ± 0·06	7·57 ± 0·20
BD + 0·3% CPIB	5·67 ± 0·30	7·21 ± 0·68
BD + 0·15% 2,4-D	7·70 ± 0·34	11·80 ± 1·19

* Animals received the respective diets (low iodine) for 2 weeks prior to the uptake measurements; 0·1 μc of carrier-free ^{131}I-iodide was given s.c. per 100 g body wt. Values are expressed as arithmetic mean ± S.E.

response.[2] Osorio *et al.*[4] showed that the liver concentrates T_4 when CPIB is given and our present data confirm this observation. It should be noted that tissue radioactivity was not positively identified as ^{131}I-T_4 because Hasen *et al.*[11] found in related studies involving the interchange of labeled thyroxine between plasma and tissues that 90–95 per cent of the tissue radioactivity was present as T_4. Heart and skeletal muscle do not concentrate T_4 (Figs. 2 and 3) but the kidney does, to a small extent. Therefore, heart and skeletal muscle show no GPD response to CPIB[2] because there is no accumulation of T_4 by these tissues, and the kidney responds slightly because of a slight increase in T_4 concentration. Other data (besides the preferential liver GPD response already discussed) also support the concept that the liver of a CPIB-treated animal becomes "hyperthyroid" in terms of its metabolism. For example, the numbers of mitochondria are increased,[4] liver slice QO_2 is increased,[2] the synthesis of new GPD enzyme is induced and liver GPD activity levels are comparable to those produced by injecting 4–8 μg T_4/100 g body weight per day,[2] liver glycogen is depleted[5] and increased protein synthesis takes place.[5] The only change not consistent with a hyperthyroid liver is its increased size. The livers of hyperthyroid rats do not become enlarged (unpublished observations).

The increase in liver metabolism, brought about presumably by the preferential uptake of T_4, is accomplished without any change in the metabolic state of the whole animal. Thorp[12] reported earlier that the rate of oxygen consumption and the respiratory quotient of CPIB-treated rats were decreased 5–10 per cent, but our current data (Table 2) show no changes in oxygen consumption rate. Furthermore, CPIB produced no demonstrable increase in the GPD activity of other tissues,[2] which suggests that these tissues remained "euthyroid" during CPIB treatment. However, the plasma PBI was reduced from 5·2 to 3·7 μg/100 ml (Table 2), but this also occurred without any increase in thyroid function (Table 3). The explanation for all of these observations may be linked to the plasma "free T_4" concentration. The T_4 concentrated by the liver appears to come from the pool of protein-bound T_4 in the plasma (Fig. 1). Although plasma T_4 is also in equilibrium with that found in other tissues, it might soon be exhausted because of the increased T_4 turnover rate seen in CPIB-treated animals if it were not for the daily endogenous T_4 supply of approximately 1 μg/100 g body weight.[13] However, CPIB appeared to change the relative percentage of plasma T_4 as "free T_4" by about 40 per cent (Table 2) and to keep its absolute concentration unchanged from control levels (2·5 mμg/100 ml plasma). The shift of a larger percentage of protein-bound T_4 to "free T_4" could explain the faster turnover of ^{131}I-T_4

and might also explain why the liver concentration of T_4 is increased. The liver contains the necessary machinery for inactivating and catabolizing T_4 and teleologically it would be the logical organ to remove excess amounts of "free T_4" from the circulation. Furthermore, if the pituitary-thyroid feedback control mechanism is controlled by the concentration of "free T_4" reaching the sensitive sites rather than by the total T_4 concentration,[14] then the unchanged absolute plasma "free T_4" concentration might explain why the animal remained euthyroid, the metabolic rate remained unchanged and the thyroid uptake value was unaffected.

Compared to the action of CPIB, the drug 2,4-D produced a much larger drop in PBI concentration (down to 2·2 μg/100 ml) and caused the thyroid to become more active as evidenced by a larger uptake of ^{131}I-iodide (Table 3). These observations are in agreement with those of Florsheim *et al.*,[15] who also found that displaced T_4 was concentrated by the liver. 2,4-D was not fed at 0·3 per cent of the diet because of its toxicity, but the 0·15 per cent dose level induced a GPD response equal to that evoked by the same dose of CPIB (Table 2). Therefore, the actions of CPIB and 2,4-D may actually be rather similar, but the effect of 2,4-D in displacing plasma T_4 is much more severe and even lowers the PBI to a point where the thyroid gland must work harder. However, there is no immediate explanation as to why 2,4-D does not produce a larger GPD response than CPIB.

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