INFLUENCE OF HORMONES ON LIVER*

I. Effects of Steroids and Thyroxine on Pyridine Nucleotide-Linked Dehydrogenases

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In the experiments now to be described, the quantitative activity of 3 soluble pyridine nucleotide-linked dehydrogenases in liver of the rat was studied before and after modifications of the endocrine status of the animal. The concentration of each of these enzymes in liver was influenced by the withdrawal of hormonal sources and also by the administration of hormones; the level of other hepatic enzymes was unmodified by these procedures. Many big effects were observed. The effective hormones were L-thyroxine and, under appropriate conditions, certain steroids of gonadal origin.

Glucose-6-phosphate dehydrogenase (1) and 6-phosphogluconic dehydrogenase (2) operate dynamically in adjacent positions in the Warburg-Lipmann-Dickens pathway (1-4) of glucose catabolism. There have been earlier indications that the levels of both enzymes are under hormonal control. The present work will show that, in liver, the response to hormones of these proximate enzymes is not unitary—certain modifications of the endocrine state which profoundly influenced the level of 6-phosphogluconic dehydrogenase had no effect on glucose-6-phosphate dehydrogenase. The concentration of lactic dehydrogenase[1] was considerably influenced by the administration and by the withdrawal of hormones of the thyroid gland. The evidence demonstrates that the liver is a singular target organ for hormones.

One of the considerations leading to the present experiments was that hydrogen atoms and electrons at critical sites of the steroid molecule are of decisive importance in the promotion of cell growth (5) by compounds exerting androgenic or estrogenic effects. The steroid groups essential for cell growth

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[1] The following abbreviations are used: 6-PGD, 6-phosphogluconic dehydrogenase; G-6-PD, glucose-6-phosphate dehydrogenase; ICD, isocitric dehydrogenase; LAD, lactic dehydrogenase; *tris*, *tris*(hydroxymethyl)aminomethane; TPN, triphosphopyridine nucleotide; DPNH, dihydrodiphosphopyridine nucleotide; DHT, dihydrotestosterone; ACTH, adrenocorticotrophin.

Thyroidectomy signifies thyroidectomy *plus* parathyroidectomy.

of these sorts are oxygen functions of two kinds, phenols or secondary alcohols, and the divergent chemical properties of these functions require, theoretically, 2 biochemical mechanisms for the promotion of cellular growth. Evidence has been accumulated which has elucidated ways in which simple hydrogen atoms and electrons at specific steroid sites can exert vast effects on biological systems. Williams-Ashman (6) studying model enzyme systems, found that phenolic steroids can act as hydrogen carriers by virtue of the reaction, phenol $\rightleftharpoons$ phenoxy free radical, promoting the oxidation of DPNH. Secondly, Talalay and Williams-Ashman discovered that soluble enzyme preparations of placenta (7, 8) and liver (9) promote the transfer of hydrogen, reversibly, from TPNH to DPN in the presence of minute amounts of steroids bearing secondary alcohol groups. Such transhydrogenation of pyridine nucleotides by enzymes in the presence of catalytic concentrations of steroid hormones made it desirable to investigate the level of the pyridine nucleotide-linked enzymes and to study the effects of hormones on these levels: hepatic enzymes were studied in the present experiments.

Another consideration underlying the present work was the higher incidence of hepatic disease, cirrhosis and cancer especially, in males than in females, in the rat (10) and in man (11). What biochemical differences between male and female liver exist to explain sex-linked predominance of hepatic diseases?

Glock and McLean (12) observed that the levels of G-6-PD and 6-PGD in rat liver are consistently higher in females than in males and they (13) "suggest that the levels of activity of both dehydrogenases are correlated with female sex hormones." Glock and McLean (14) also discovered that there is a very significant increase in the levels of activity of both dehydrogenases in the livers of rats following injections of thyroxine, the increase being "approximately twofold." The administration of thiouracil (14) caused a decreased concentration of 6-PGD and G-6-PD in liver but did not significantly affect total liver values. It has been reported that hypophysectomy has no effect on the levels in rat liver of 6-PGD (15) or G-6-PD (15, 16).

Chatagner and Bergeret studied the effects of hormones upon the levels of pyridoxal phosphate-linked decarboxylases in liver. With reference to values in the liver of intact females, ovariectomy (17) or thyroidectomy (18) resulted in an *increase* in the rate of decarboxylation of cysteine sulfinic acid; these augmented rates were lowered, respectively, by the administration of estradiol-17β (17) or thyroxine (18). In comparison to the liver of males, the female liver contains greater amounts of non-specific cholinesterase (19) and β-glucuronidase (20); but female liver has lesser activities of catalase (21) and cysteic acid decarboxylase (22).

Methods

Albino rats of Sprague-Dawley strain were obtained from the dealer at age 42 days and kept thereafter in a controlled climate; the rats seemed to be free from infectious disease during the experiments. Most of the rats were fed a commercial ration; hypophysectomized rats were kept on a synthetic high protein (18 per cent) diet (23) consumed *ad libitum*; adrenalectomized rats were maintained by providing physiological saline as drinking fluid. All operations were carried out under ether anesthesia. Experiments on rats from which endocrine glands had been removed were not begun until 3 weeks after the operation.

Most of the steroids were dissolved in ethyl alcohol and diluted with sesame oil to make the final alcoholic concentration 10 per cent. The solution (0.2 ml.) was injected intramuscularly daily. Cortisone acetate was injected subcutaneously in the form of a suspension in saline. Protein hormones[2] and L-thyroxine were dissolved in 0.1 N sodium hydroxide and diluted with saline. *Throughout this paper dosage refers to the amount administered each day.*

Preparation of Enzymes.—The animals were killed by decapitation. The organs for analysis were excised rapidly, weighed, and homogenized for 3 minutes in an ice-cold solution of 0.15 N NaCl containing 0.003 M $NaHCO_3$; the enzyme solutions were kept in an ice bath thereafter until the assays were made. The homogenates were centrifuged at 11,000 *g* for 15 minutes in a refrigerated centrifuge. The supernatant solutions were not dialyzed and assays of enzymes were carried out on them without delay. The nitrogen content of the organs was determined by a micro-Kjeldahl technique.

Enzyme Assays and Units.—Spectrophotometric determinations were made with a Beckman model DU spectrophotometer at 25°C. in an air-conditioned room using either pyrex or silica cells of 1 cm. light path. The reduction (or oxidation) of pyridine nucleotides was followed by measurement of change in absorbence at 340 mμ. The molar extinction coefficient of reduced pyridine nucleotides at 340 mμ was assumed to be 6,220. Only the initial velocity of reduction of TPN was measured using an amount of enzyme solution which reduced TPN at a rate not exceeding 0.007 micromoles/minute; these conditions yielded zero order kinetics for all of the dehydrogenases.

The assay of 6-PGD was based on the method of Glock and McLean (12). The reaction mixture consisted of:—

0.5 M *tris*, pH 7.4	0.5 ml.
0.5 M $MgCl_2$	0.1 "
0.005 M TPN	0.1 "
0.05 M sodium 6-phosphogluconate	0.1 "
Distilled water	2.15 "
Enzyme solution (*ca.* 0.3 mg. liver)	0.05 "

The assay of G-6-PD was a modification of the double substrate method of Glock and McLean (12). The enzyme solution was not dialyzed. The reaction mixture consisted of:—

0.5 M *tris*, pH 7.4	0.5 ml.
0.5 M $MgCl_2$	0.1 "
0.005 M TPN	0.1 "
0.05 M sodium 6-phosphogluconate	0.1 "
0.1 M disodium glucose-6-phosphate	0.1 "
Distilled water	2.05 "
Enzyme solution (*ca.* 0.3 mg. liver)	0.05 "

One unit of 6-PGD or of G-6-PD is defined as the enzyme activity which reduced 1 μ mole of TPN/1 minute under the stated conditions. The units are expressed in terms of 1 gm. of fresh tissue (wet weight).

The assay of LAD was adapted from Kubowitz and Ott (24). The reaction mixture consisted of:—

0.5 M *tris*, pH 7.4	0.5 ml.
0.001 M DPNH	0.3 "
0.1 M sodium pyruvate	0.1 "
Distilled water	2.05 "
Enzyme solution (*ca.* 0.1 mg. liver)	0.05 "

[2] Bovine pituitary growth hormone and ovine lactogenic hormones were generously donated by Professor C. H. Li, University of California, Berkeley.

One unit of LAD is defined as the enzyme activity which oxidized 1 μ mole of DPNH/1 minute under the stated conditions. The units are expressed in terms of 1 gm. of fresh tissue (wet weight).

Acid phosphatase (pH 5.1) and alkaline phosphatase (pH 9.3) were determined by the method of King and Armstrong (25) and are expressed in the units of these authors per gram of fresh tissue (wet weight).

TABLE I

Dehydrogenases in Liver in Early Life

The results are expressed in units per gram, wet weight; mean values are given. All of the rats were females.

No. of rats	Dehydrogenases		
	6-Phosphogluconic	Glucose-6-phosphate	Lactic
	Fetus: day 18 of pregnancy		
4	2.29	1.84	113
	Fetus: day 22 of pregnancy		
8	3.88	1.43	121
	Females: age 3 days		
8	3.49	1.70	329
	Females: age 15 days		
8	2.38	1.44	570
	Females: age 20 days		
4	3.06	1.01	275
	Females: age 49 days		
4	4.78	1.64	214

EXPERIMENTAL

Reproducibility of Methods.—In an earlier study (12), the activities of 6-PGD and G-6-PD were determined on dialyzed liver extracts. We repeated this experiment on enzyme activities in dialyzed and undialyzed extracts. Dialysis always resulted in decreased enzymatic activity of 6-PGD and of G-6-PD, the dialyzed samples yielding results 15 to 34 per cent lower than the undialyzed extracts. For this reason dialysis was not performed in the present experiments.

In experiments on reproducibility of results, enzyme activities were determined on 10 samples selected at random from a single liver; the enzyme assays were carried out promptly and mean values, with standard deviation, of the results were calculated. In one experiment, typical of many, the standard devia-

tions (expressed in percentage of the mean) were: 6-PGD, ± 7.0 per cent; G-6-PD, ± 8.4 per cent; LAD, ± 5.0 per cent. It was concluded that the reproducibility of the methods was within 10 per cent of the mean values when extracts of different areas of a liver were prepared and assayed.

TABLE II

Effects of Extirpation of Certain Endocrine Glands on Dehydrogenases in Liver

The results are expressed in units per gram of liver, wet weight. Surgical operations were performed 3 weeks before the enzymes were studied.

No. of rats	Dehydrogenases		
	6-Phosphogluconic	Glucose-6-phosphate	Lactic
		Females: normal,* age 56–70 days	
20	7.57 ± 1.0	4.37 ± 1.4	260 ± 55
		Females: ovariectomy	
20	5.14 ± 0.7	3.02 ± 1.0	247 ± 49
		Females: adrenalectomy*	
10	5.54 ± 1.0	2.20 ± 0.6	252 ± 18
		Females: thyroidectomy*	
28	2.25 ± 0.3	0.88 ± 0.2	131 ± 17
		Females: thyroidectomy and ovariectomy	
10	2.30 ± 0.8	0.86 ± 0.2	157 ± 14
		Females: hypophysectomy	
20	2.62 ± 0.2	0.88 ± 0.2	211 ± 20
		Males: normal, age 56–70 days	
15	4.51 ± 0.8	1.08 ± 0.3	285 ± 31
		Males: orchiectomy	
14	4.62 ± 1.0	1.29 ± 0.3	244 ± 38

±, Standard deviation.
* Rats in estrus.

Levels of Enzymes in Liver in Early Life.—The concentration of pyridine nucleotide-linked dehydrogenases in fetal liver was determined from days 18 to 22 of pregnancy; low levels of all of these enzymes were found (Table I). The values rose after birth but each enzyme increased at a rate different from the others.

The activity of LAD was unique. At age 3 days, the level of LAD in liver rose to the considerable height of 329 units/gm. The concentration of this

enzyme increased progressively until age 15 days when a mean value of 570 units/gm. was found; this level, obtained while the animals were maintained exclusively on mother's milk, was notably higher than any other encountered in the present experiments. After 15 days, nourishment was obtained from solid food in the cage and from suckling; at age 20 days, the concentration of LAD in liver had declined and it had reached the level characteristic of normal adult rats (Table II).

The concentration of 6-PGD and G-6-PD in the liver of young females

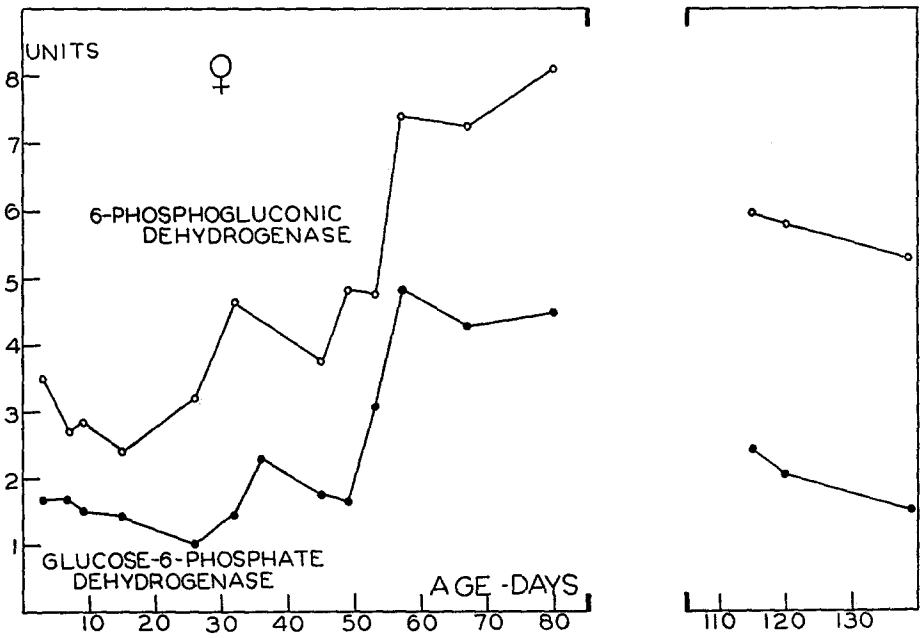

FIG. 1. The rise in hepatic concentration of 6-PGD and G-6-PD in normal female rats during adolescence and the relative decline in concentration at age 114 to 138 days.

remained at the low levels characteristic of fetal liver for weeks—6-PGD until age 26 days and G-6-PD until age 53 days; after these ages, the values increased rapidly and considerably (Fig. 1). These high values of early adult life did not persist; at age 4 months, a decline in concentration of 6-PGD and, especially, of G-6-PD was observed in female liver (Fig. 1). No rise of 6-PGD or G-6-PD was observed in the liver of male rats during puberty.

Effects of Extirpation of Endocrine Glands on Hepatic Enzymes

The concentration of 6-PGD in liver in all of the experiments always exceeded that of G-6-PD except in mothers in the middle of the period of lactation, a matter to be discussed below.

The concentrations of LAD found in livers of males and females were of a similar order of magnitude and gonadectomy did not influence these values. Hypophysectomy resulted in a slight decrease in concentration whereas thyroidectomy induced a great decline in the level of LAD (Table II).

Relatively high levels of 6-PGD and G-6-PD were found in the livers of young adult female rats (Table II). The removal of the ovaries or the adrenals

TABLE III

Dehydrogenases in Liver and Other Organs after Hypophysectomy

The results are expressed in units per gram, wet weight. Mean values are given with 4 adult female rats in each group. Hypophysectomy had been performed 3 to 4 weeks before enzyme assays.

Hormonal status	Dehydrogenases		
	6-Phosphogluconic	Glucose-6-phosphate	Lactic
		(*a*) Liver	
Intact	6.76	5.37	285
Hypophysectomy	3.37	1.01	228
		(*b*) Renal cortex	
Intact	1.99	2.20	124
Hypophysectomy	2.28	2.76	138
		(*c*) Spleen	
Intact	2.26	6.40	101
Hypophysectomy	3.43	6.36	106
		(*d*) Heart	
Intact	0.57	0.26	281
Hypophysectomy	0.51	0.40	285
		(*e*) Lung	
Intact	1.72	3.23	56
Hypophysectomy	1.80	4.32	65

resulted in a moderate lowering of concentration of both enzymes; hypophysectomy reduced their content greatly. The effect of removal of the thyroid gland on the hepatic concentration of G-6-PD was profound—the level of G-6-PD fell to that found in livers of hypophysectomized rats. The total content of 6-PGD and G-6-PD in the livers of thyroidectomized or hypophysectomized rats was of the order of one-fifth of that in adult intact sisters.

Higher concentrations of 6-PGD and G-6-PD in liver were found in females than in males. The very low concentration of G-6-PD in male liver is remarkable; it approximated the activity of G-6-PD found in livers of females deprived

of the pituitary or thyroid gland. In contrast to the effects in the female, gonadectomy did not alter the levels of 6-PGD and G-6-PD in the male.

Hormonal Effects Operative in Liver but Not in Certain Other Organs

Whereas hypophysectomy was followed by a considerably decreased concentration of 6-PGD and G-6-PD in liver, the level of LAD was little affected by this procedure. In contrast to the profound effects of hypophysectomy on

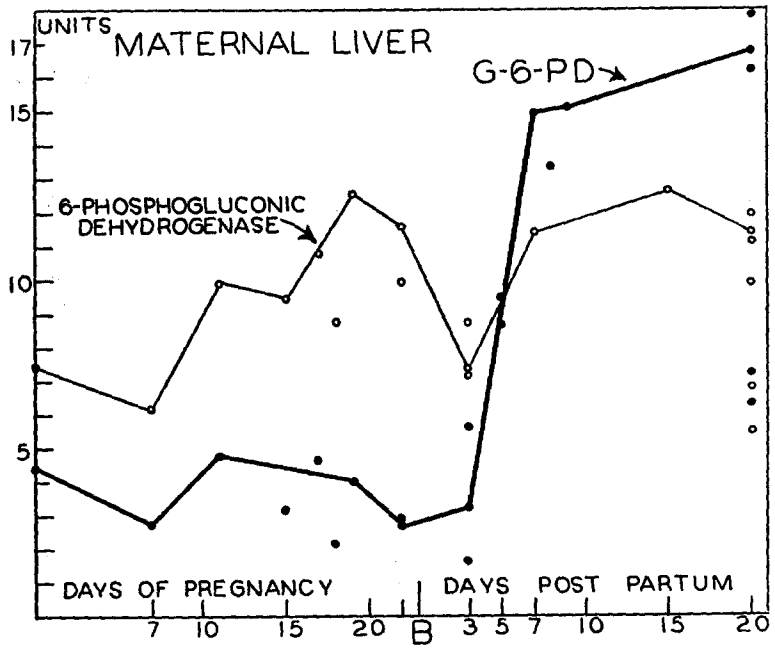

FIG. 2. During pregnancy the hepatic level of 6-PGD rose in maternal liver whereas the concentration of G-6-PD remained unchanged. Shortly after day 3, post partum, the concentration of G-6-PD rose to very high heights. *B*, the day of birth.

the dehydrogenases of the hexose monophosphate shunt in liver, removal of the pituitary was not followed by a decrease in the concentration of 6-PGD, G-6-PD, or LAD in renal cortex, spleen, heart, or lung (Table III); instead a slight increase in concentration of the dehydrogenases was usually observed in these organs. These findings demonstrate some specificity for hormonal effects on the liver.

Effects of Pregnancy and Lactation.—The finding of spermatozoa in the vagina dated the onset of pregnancy. The level of hepatic 6-PGD rose steadily during pregnancy. After birth, there was a decrease in concentration for several days but high values of 6-PGD were again found from days 5 to 15 post partum in the livers of all nursing mothers and until weaning in most of them (Fig. 2).

The concentration of G-6-PD did not rise during pregnancy and it remained moderately low through day 3 post partum but between days 3 and 5 it started to rise to higher levels. Very high levels of G-6-PD (Fig. 2) were found during midlactation in all of the nursing mothers and until day 20 (the day of weaning in many of them. Lactation was the only state encountered in the present study in which G-6-PD exceeded 6-PGD in concentration. During lactation, uterus and vagina underwent progressive involution, reaching the pregravid state on

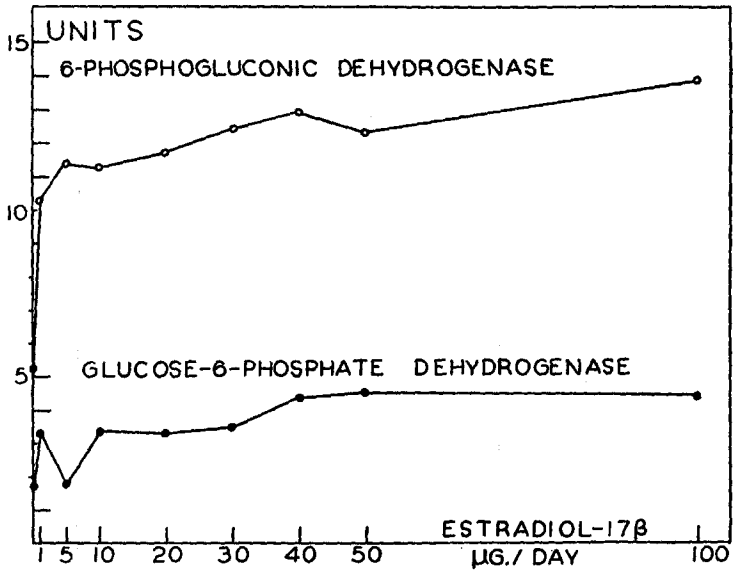

FIG. 3. The administration of estradiol-17β for 21 days to ovariectomized female rats resulted in a marked increase in the hepatic concentration of 6-PGD while the level of G-6-PD remained unchanged. Estradiol-17β, in dosage from 1 to 100 μg. was administered to groups of rats and mean values are plotted.

post-partum day 20; throughout lactation the mammary glands continued to grow and to produce ever increasing amounts of milk.

Effects of Estradiol-17β.—Estradiol-17β was administered daily to groups of ovariectomized rats for 21 days; the dosage for each group was not altered during the period of injection. The hepatic concentrations of LAD remained unmodified by the administration of estradiol-17β at all dose levels; the effect of the hormone on the concentration of 6-PGD was far different.

A pronounced rise in the hepatic level of 6-PGD in gonadectomized rats, both male and female, followed the administration of estradiol-17β, 1 μg., for 3 weeks; a daily dosage of 0.1 μg. was ineffective. Increasing the dose of estradiol-17β from 1 to 100 μg. augmented (Fig. 3) the concentration of 6-PGD in liver but only to a slight extent.

A study was made of the relation of time to the rise of 6-PGD in liver of ovariectomized rats; 2 levels of dosage of estradiol-17β, 20 and 50 μg., were selected but the difference in dose did not influence the results. The hepatic level of 6-PGD was not changed significantly for 7 to 11 days after the inception of administration of estradiol-17β, 20 μg., but between days 11 and 13 (Fig. 4), there was a decided rise in the liver of every rat and the enzyme values remained high throughout the remainder of the experiment.

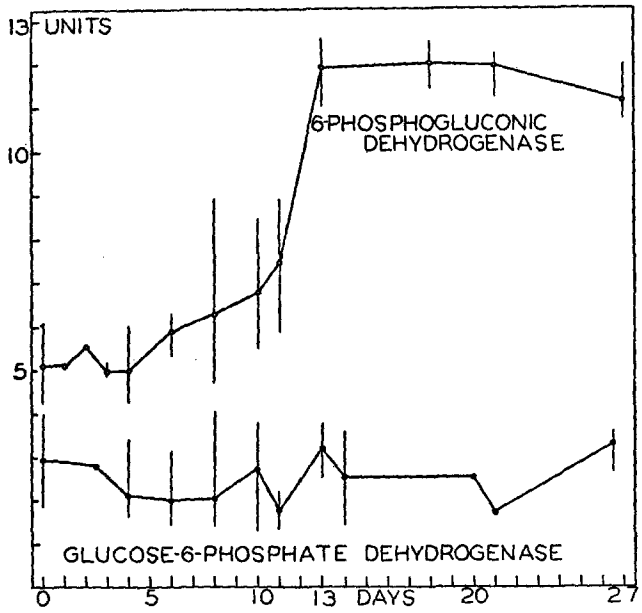

FIG. 4. Increase in hepatic concentration of 6-PGD induced by the daily administration of estradiol-17β, 20 μg., related to duration of this treatment. The hepatic concentration of G-6-PD remained unchanged. Mean values and the extreme ranges in concentration are given.

A sex difference was found in the influence of estradiol-17β on the hepatic level of G-6-PD. In gonadectomized females the level of G-6-PD in liver was not modified by the administration of estradiol-17β (Figs. 3 and 4). In castrate males the concentration of G-6-PD in liver increased approximately 4-fold following the injection of estradiol-17β, 20 μg., for 3 weeks; mean values of G-6-PD/gm. liver in uninjected males were 1.22 units whereas in estradiol-treated brothers the concentration was 5.32 units.

Effect of Partial Hepatectomy.—Removal of the median and left lateral lobes of the liver (*ca.* 70 per cent of total hepatic weight) was performed in 2 groups of ovariectomized rats. The one group had been treated before hepatectomy

with estradiol-17β, 20 μg., for 3 weeks to induce elevated levels of 6-PGD; these animals received the hormone for 14 days subsequent to hepatectomy. The other group received no hormones. The concentration of dehydrogenases was determined at intervals in both groups.

Similar patterns of activity of 6-PGD, but with quantitative differences, were observed in the regenerating liver of both groups. The concentration of 6-PGD declined progressively for 5 days and pre-operative levels were reached 11 days after hepatectomy. Notably, in the livers of rats treated with estradiol-17β, the concentration of 6-PGD was rapidly restored to the high pre-operative

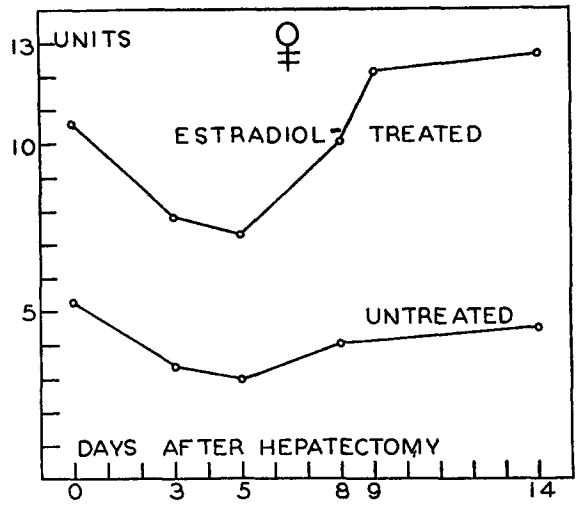

FIG. 5. Influence of partial hepatectomy on the concentration of 6-PGD in regenerating liver of ovariectomized rats. One group, injected with estradiol-17β, 20 μg., for 3 weeks before hepatectomy received this hormone after hepatectomy as well. Hormones were not administered to the second group.

level (Fig. 5); in the untreated group of ovariectomized rats recovery occurred to the low level prevailing before hepatectomy. The regenerating daughter liver cells reproduced the 6-PGD level of the mother cells.

Hormonal Effects in Adrenalectomized-Ovariectomized Rats.—The levels of pyridine nucleotide-linked dehydrogenases in the liver of adrenalectomized rats were similar to those of ovariectomized animals (Table II).

Groups of rats subjected to adrenalectomy plus ovariectomy were injected with hormones. Cortisone, 0.1 mg., did not alter the levels of 6-PGD or G-6-PD in liver and the rats gained weight during the experimental period. The larger doses of cortisone used in the experiment resulted in a pronounced loss of body weight. The injection of cortisone, 0.5 mg., caused an increase in the concentration of 6-PGD without altering the level of G-6-PD; larger doses of

cortisone, 1 to 2 mg., did not increase the concentration of 6-PGD additionally but did induce a moderate elevation of G-6-PD (Table IV). Elevated values of 6-PGD and G-6-PD in cortisone-treated rats were observed solely in animals in which cortisone had caused a great loss of weight.

Hormonal Effects in Liver of Hypophysectomized Rats.—The administration of estradiol-17β to hypophysectomized rats failed to alter the concentration of dehydrogenases in liver (Table V). Other hormones were administered separately or in combination with estradiol-17β without modifying the level of the hepatic enzymes; hormones ineffective in this regard, with their dosage, are:

TABLE IV

Effects of Hormones on Enzymes in Liver of Adrenalectomized-Ovariectomized Rats

All of the rats were operated upon 4 weeks prior to starting injections. Rats receiving cortisone drank water; the others received saline as drinking fluid. Hormones were administered daily for 3 weeks with 4 rats in each group. Mean values expressed in units per gram (wet weight) are given. Δ is the change in body weight during the experiment.

Hormones, dosage	Δ	Dehydrogenases	
		6-Phosphogluconic	Glucose-6-phosphate
	gm.		
None	+50	4.65	2.34
Cortisone, 0.1 mg.	+14	3.95	1.49
Cortisone, 0.5 mg.	−50	5.02	1.79
Cortisone, 1 mg.	−35	6.27	4.35
Cortisone, 2 mg.	−40	6.02	4.86
Estradiol-17β, 10 μg.	+48	8.73	4.75
Estradiol-17β, 10 μg., and cortisone, 2 mg.	+8	11.73	6.83

cortisone, 0.25 mg.; progesterone, 3 mg.; pituitary growth hormone, 0.5 mg.; lactogenic hormone, 0.5 mg.; ACTH, 4 units.

But the administration of L-thyroxine, 10 μg., was highly effective in hypophysectomized rats in increasing the concentration and total hepatic content of 6-PGD, G-6-PD and LAD. L-Thyroxine caused an increase in these 3 hepatic dehydrogenases without modifying significantly the concentration of acid phosphatase or alkaline phosphatase.

Hormonal Effects in Rats Subjected to Thyroidectomy and Ovariectomy

Dehydrogenases of liver were studied in rats deprived of thyroid gland and ovaries and injected with hormones. In thyroidectomized-ovariectomized rats, the concentration of 6-PGD, and especially the levels of G-6-PD and LAD, were low. In rats of this class, estradiol-17β did not influence the level of the hepatic enzymes (Table VI). But L-thyroxine, 100 or 500 μg. caused a decided

TABLE V

Effects of Hormones on Enzymes of Liver of Hypophysectomized Rats

All of the rats were subjected to hypophysectomy 4 weeks before starting the injections. Hormones were administered daily for 3 weeks with 4 rats in each group. Mean values expressed in units per gram, wet weight, are given.

Hormones, dosage	Dehydrogenases			Phosphatases	
	6-Phospho-gluconic	Glucose-6-phosphate	Lactic	Acid	Alkaline
None	2.68	0.80	242	11.9	0.85
Estradiol-17β, 10 μg.	2.68	0.81	216	11.8	0.87
L-Thyroxine, 10 μg.	8.63	2.36	298	11.1	0.91
L-Thyroxine, 100 μg.	9.11	1.87	292	12.8	0.99
Estradiol-17β, 10 μg., and L-thyroxine, 10 μg.	7.62	2.06	292	12.1	0.93

TABLE VI

Hormonal Effects on Dehydrogenases of Liver of Rats Deprived of Ovaries and Thyroid Gland

All of the rats were subjected to ovariectomy and thyroidectomy 2 months before starting the injections. Hormones were administered daily for 3 weeks, with 4 rats in each group. Mean values are given.

Dehydrogenases				
Units/gm. wet weight			Units/total liver	
6-PGD	G-6-PD	Lactic	6-PGD	G-6-PD
		Controls: uninjected		
2.2	1.0	154	29.8	7.2
		Estradiol-17β, 20 μg.		
2.6	0.8	189	32.7	5.9
		L-Thyroxine, 0.1 mg.		
7.6	2.4	311	55.4	17.3
		L-Thyroxine, 0.5 mg.		
8.2	3.8	318	61.9	26.8
		L-Thyroxine, 0.5 mg. and estradiol—17β, 20 μg.		
11.7	4.4	352	106.2	39.3
		L-Thyroxine, 0.5 mg. and DHT*, 1 mg.		
7.2	2.7	308	61.7	22.8

*DHT, dihydrotestosterone.

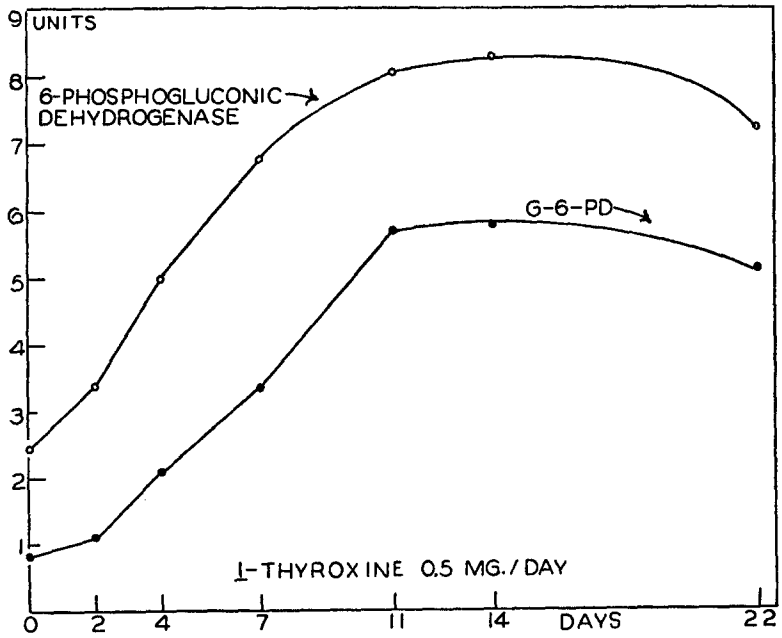

FIG. 6. Rise in hepatic concentrations of 6-PGD and G-6-PD resulting from the administration of L-thyroxine, 0.5 mg., to rats deprived of ovaries and thyroid glands 8 weeks earlier.

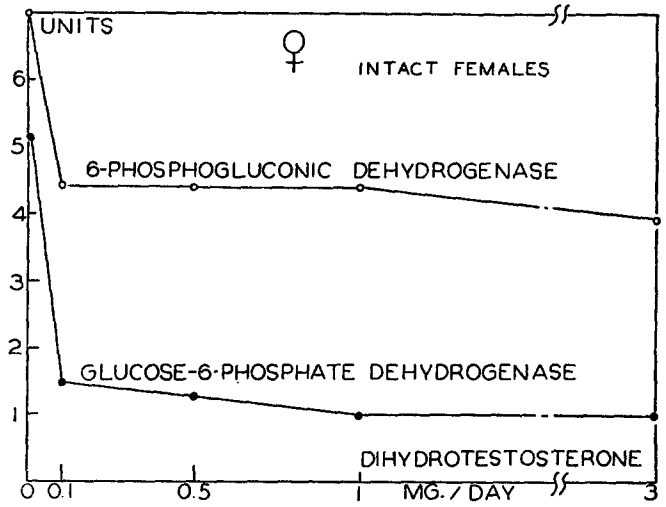

FIG. 7. The administration of dihydrotestosterone, 0.1 to 3 mg. daily for 3 weeks, induced a moderate decline in the hepatic level of 6-PGD and a profound decrease in the concentration of G-6-PD.

increase in the content and concentration in the liver of 6-PGD, G-6-PD, and LAD; the effects of smaller quantities of L-thyroxine were not investigated.

L-Thyroxine, 0.5 mg., restored the sluggish body growth which prevails in thyroidectomized rats to the growth rate of normal sisters; the atrophic adrenal glands, characteristic of thyroid deficiency, likewise were restored to normal size within 3 weeks. The liver is brown in exsanguinated thyroidectomized rats. In mates injected with L-thyroxine for 2 days or more, the liver quickly turns from dark brown to red or purple on exposure to air.

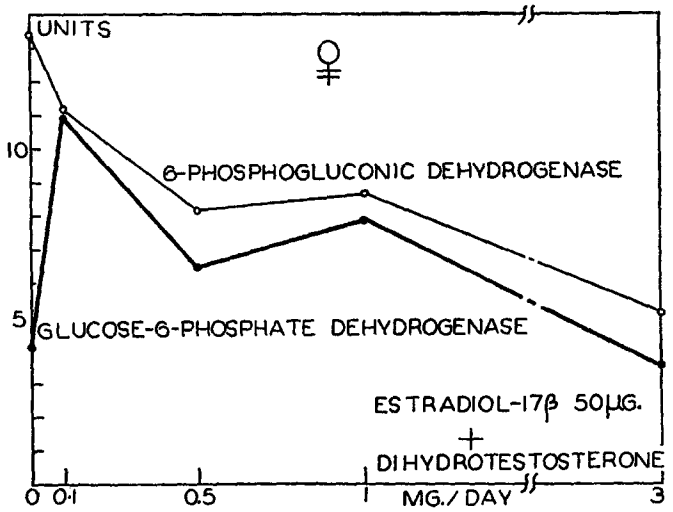

FIG. 8. Effects on the hepatic levels of 6-PGD and G-6-PD of the concurrent administration of estradiol-17β, 50 μg., and dihydrotestosterone (at various dose levels) to ovariectomized rats. The level of 6-PGD, which was elevated in rats treated with estradiol-17β alone, declined progressively with increasing doses of dihydrotestosterone. The daily dose of estradiol-17β, 50 μg., and dihydrotestosterone, 1 mg., caused a pronounced elevation of G-6-PD.

A quantitative study was made of the effects of daily injections of L-thyroxine, 0.5 mg., on the concentration of 6-PGD and G-6-PD in livers of rats deprived of ovaries and thyroid glands; groups of these rats were killed at intervals. L-Thyroxine, 0.5 mg., caused the animals to gain weight, on average 2 gm. daily. L-Thyroxine resulted in rapid elevation of 6-PGD, demonstrated unequivocally 4 days after the first injection (Fig. 6). The concentration of G-6-PD also increased in the livers of sprayed rats injected with L-thyroxine, in contrast to the findings in ovariectomized rats in which no rise of G-6-PD followed the administration of estradiol-17β.

Effects of Androstane Derivatives on Hepatic Enzyme Levels.—Young adult females were injected with dihydrotestosterone for 3 weeks; the hepatic level of 6-PGD (Fig. 7) was reduced somewhat compared with the finding in un-

treated sisters whereas a profound decrease in the concentration of G-6-PD (Fig. 7) resulted from the administration of dihydrotestosterone.

Earlier in this paper, it was demonstrated that in ovariectomized rats estradiol-17β induced a great increase in concentration of 6-PGD in liver whereas the level of G-6-PD was unchanged; in castrate males estradiol-17β resulted in markedly increased hepatic levels of both 6-PGD and G-6-PD. The combined administration of dihydrotestosterone and estradiol-17β prevented the rise in the level of 6-PGD and the inhibitory effect of dihydrotestosterone was stoichiometric (Fig. 8). Unexpectedly, the combined administration of estradiol-17β and moderate doses of dihydrotestosterone resulted in a pronounced increase in the level of G-6-PD in liver of spayed females (Fig. 8). Other 3α-hydroxysteroids (androstane-3α, 17β-diol; 4-androsten-3α, 17β-diol) exerted the same influence on hepatic enzymes as dihydrotestosterone did.

In contrast to the androstane derivatives, cortisone, 2.5 mg. or progesterone, did not block the estradiol-induced rise of 6-PGD in liver.

Dihydrotestosterone did not inhibit the effects of L-thyroxine in inducing elevation of 6-PGD and G-6-PD in liver (Table VI).

DISCUSSION

The liver is in a special category as a target organ in its responses to hormonal modifications of the *milieu intérieur*. The concentrations of 3 hepatic dehydrogenases requiring pyridine nucleotides as hydrogen acceptors were influenced considerably by endocrine changes. Not all organs were similarly responsive; *e.g.*, hypophysectomy caused a considerable decrease in the concentrations of 6-PGD and G-6-PD in liver but had little effect on their levels in kidney, spleen, heart, or lung. Not all hepatic enzymes were influenced by hormonal modifications; often the concentrations of acid, and of alkaline phosphatase were uninfluenced by hormonal effects which profoundly modified levels of dehydrogenase activities.

It was learned in the present experiments that multiple endocrine factors influence the levels of hepatic dehydrogenases; of these hormones, thyroxine and steroids both in the C_{18} and in the C_{19} series have high significance. The multiplicity of factors accounts for the seemingly complex observations.

Glock and McLean (14) discovered that thyroxine influenced the level of 6-PGD and G-6-PD in liver, an observation confirmed by us. In the present work, it was observed that steroids in the androstane and estrane series exert powerful and, under stated conditions, opposing effects on the levels of G-6-PD and 6-PGD in liver.

Each of 3 hepatic dehydrogenases, LAD, 6-PGD, and G-6-PD increased in an individually characteristic pattern from the relatively low levels of late fetal life. The concentration of LAD had risen considerably at age 3 days; the concentration of isocitric dehydrogenase has been found (26) to increase in a

similar manner. In the case of LAD the highest values observed in the present experiments were found at age 15 days, but at age 20 days, the level characteristic of adult rats was found. It would appear that this rise of LAD in early life was determined primarily by metabolic causes.

After birth, the hepatic concentration of 6-PGD remained at the low level of fetal liver until age 26 days; then in female rats, but not in males, a gradual increase of concentration occurred until high values were found at age 57 days. Likewise in female rats, the hepatic concentration of G-6-PD remained at the low level of late fetal life until age 50 days when it rose considerably; no rise of G-6-PD occurred in the liver of untreated males. The developmental patterns of 6-PGD and G-6-PD appear to be primarily influenced by hormones.

The level of LAD in liver was profoundly modified by the presence or absence of thyroid hormones—by administration of L-thyroxine or by thyroidectomy. Changes in the steroid status of the animal exerted no highly significant influence on the hepatic concentration of LAD in the present experiments.

The experiments demonstrated that 2 hormones, estradiol-17β, and L-thyroxine can increase the level of 6-PGD in liver. The concentration of 6-PGD in liver of adult female rats was reduced by ovariectomy. Gonadectomy in the male was not followed by a decline in the hepatic level of 6-PGD because the testis of the rat secretes small amounts of estrogens. Hypophysectomy profoundly reduced the hepatic level of 6-PGD in both sexes.

The level of 6-PGD in liver was considerably augmented by the administration of estradiol-17β to ovariectomized rats. It is significant that this rise did not occur promptly; there was an interval of 12 days after commencing the injections before high values of 6-PGD were found in the liver of every rat. Elevated levels of 6-PGD induced by estradiol-17β were prevented by the simultaneous injection of dihydrotestosterone and by 3α-hydroxysteroids and the degree of inhibition had a stoichiometric relation to the quantity of the androstane derivative which was administered. Estradiol-17β did not induce an elevation of the level of 6-PGD in thyroidectomized or hypophysectomized rats. But the level of 6-PGD rose in livers of rats, deprived of thyroid or pituitary after the administration of L-thyroxine alone—and to specially high levels when estradiol-17β was injected together with L-thyroxine. Thyroxine-induced elevation of 6-PGD in liver was rapid—significantly elevated values were observed after 3 days in contrast to the delay following the administration of estradiol-17β.

Three hormonal factors were found to augment the concentration of G-6-PD in liver: (*a*) testosterone, (*b*) estradiol-17β, and (*c*) thyroxine; testosterone was augmentative *only* when administered with estradiol-17β. The concentration of G-6-PD in liver was higher in adult females than in males; the level was reduced somewhat by ovariectomy. But orchiectomy did not lower the hepatic level of G-6-PD in the male.

The hepatic levels of G-6-PD of adult females were much depressed by (*a*) administration of dihydrotestosterone, or (*b*) hypophysectomy, or (*c*) thyroidectomy. Remarkably, each of these procedures was equivalent in its effectiveness. Moreover, the concentration of G-6-PD in adult male liver was of the same order of magnitude as that found in hypophysectomized females.

In intact males, low hepatic values of G-6-PD are explained by the presence of effective amounts of testosterone; in castrate males the low values are due to the absence of effective quantities of phenolic estrogens or thyroxine.

Estradiol-17β did not cause an increase in the concentration of G-6-PD in overiectomized females but it was highly effective in this regard in castrate males. In spayed females, and in males as well, the hepatic level of G-6-PD was markedly increased when testosterone was administered together with estradiol-17β. But testosterone administered alone depressed the level of G-6-PD in intact females. Why, in gonadectomized rats, does estradiol-17β elevate the hepatic level of G-6-PD in males and possess no such effect in females? It would appear that the castrate male posesses a synergistic factor, akin to testosterone, which is deficient in spayed female rats: indeed, castrate males grow more rapidly after gonadectomy than spayed females do but the reason for this disparity between the sexes has not been elucidated. L-Thyroxine always induced a rise in the level of G-6-PD in the livers of rats of both sexes.

There exists a basal level of concentration of the 3 dehydrogenases in liver which must be essential for life of the cell. It was possible to effect an increase in the level of 6-PGD without promoting an increase above the minimal level of G-6-PD; the opposite was not observed—increased values of G-6-PD were always accompanied by elevated levels of 6-PGD as well.

We see that each of 3 hepatic dehydrogenases has peculiarities of response to modifications of the endocrine status of the rat and gonadal steroids and thyroid hormones are of significance in determining the hepatic levels of these enzymes. But thyroid hormones and steroids are not equivalent in effectiveness. Thyroxine is preeminent in determining the levels in the liver of LAD, G-6-PD, and 6-PGD as shown by the following evidence: (*a*) Thyroidectomy reduced the concentration of each dehydrogenase to a basal constitutive level whereas modification of the steroid status fails to modify greatly the level of LAD; (*b*) The concentration of each dehydrogenase was elevated following the administration of L-thyroxine whereas in ovariectomized females, estradiol-17β increased the concentration only one of the dehydrogenases (6-PGD) under discussion and the estrogen was ineffective in this regard in the absence of thyroid hormones.

Are there 2 physiologic mechanisms, respectively, steroid- or thyroid-dependent, for increasing the levels of 6-PGD and G-6-PD or do the great effects of the steroids on the concentration of hepatic enzymes result solely

from modifications of the rate of secretion of thyroid hormones which steroids might induce as a secondary effect? The evidence favors 2 separate mechanisms in liver for controlling the hepatic concentration of dehydrogenases. For, (*a*) thyroxine raised the levels of 6-PGD, G-6-PD, and LAD in spayed female rats whereas estradiol-17β increased the concentration of 6-PGD alone; and (*b*), dihydrotestosterone, administered concurrently, blocked the capacity of estradiol-17β to increase the concentration of 6-PGD but did not depress the augmented levels of dehydrogenases induced by thyroxine.

SUMMARY

Modification of the endocrine *milieu intérieur* by administration or withdrawal of hormones considerably influenced the concentration in liver of lactic dehydrogenase, glucose-6-phosphate dehydrogenase, and 6-phosphogluconic dehydrogenase. Hormones effective in this regard are thyroxine, estradiol-17β, dihydrotestosterone, and 3α-hydroxysteroids in the androstane series. These hormonal effects are both organ-selective and, in liver, enzyme-selective.

Thyroxine and steroids are not equivalent in their influence on the 3 hepatic dehydrogenases. Thyroxine was preeminent in this regard among hormones investigated in the present experiments.

Each of the pyridine nucleotide-linked dehydrogenases in liver had an individually characteristic response to the administration of various hormones which was reflected in its hepatic level. The concentration in liver of lactic dehydrogenase was profoundly raised or lowered, respectively, by the administration of thyroxine or by thyroidectomy, and injection of steroids had small influence on its level.

The levels of glucose-6-phosphate dehydrogenase and of 6-phosphogluconic dehydrogenase were considerably influenced by injection of thyroxine, estradiol-17β, or testosterone. Sex steroids did not increase the levels of these enzymes in hypophysectomized or thyroidectomized rats unless thyroxine was administered concurrently.

Under designated conditions, it was found that the concentration of 6-phosphogluconic dehydrogenase was increased by hormonal methods which did not elevate the concentration of glucose-6-phosphate dehydrogenase. But high levels of glucose-6-phosphate dehydrogenase were not achieved without a considerable elevation of 6-phosphogluconic dehydrogenase as well.

BIBLIOGRAPHY

1. Warburg, O., and Christian, W., Ueber Aktivierung der Robisonschen Hexosemono-phosphosäure in roten Blutzellen und die Gewinnung aktivierender Fermentlosungen, *Biochem. Z.*, 1931, **242**, 206.
2. Warburg, O., Christian, W., and Griese, A., Wasserstoffübertragendes Co-ferment, seine Zusammensetzung und Wirkungsweise, *Biochem. Z.*, 1935, **282**, 157.

3. Lipmann, F., Fermentation of phosphogluconic acid, *Nature*, 1936, **138,** 588.
4. Dickens, F., Mechanism of carbohydrate oxidation, *Nature*, 1936, **138,** 1057.
5. Huggins, C., and Jensen, E. V., Significance of the hydroxyl groups of steroids in promoting growth, *J. Exp. Med.* 1954, **100,** 241.
6. Williams-Ashman, H. G., Cassman, M., and Klavins, M., Two enzymic mechanisms for hydrogen transport by phenolic oestrogens, *Nature*, 1959, **184,** 427.
7. Talalay, P., and Williams-Ashman, H. G., Activation of hydrogen transfer between pyridine nucleotides by steroid enzymes, *Proc. Nat. Acad. Sc.* 1958, **44,** 15.
8. Talalay, P., Hurlock, B., and Williams-Ashman, H. G., On a coenzymatic function of estradiol-17β, *Proc. Nat. Acad. Sc.* 1958, **44,** 862.
9. Hurlock, B., and Talalay, P., 3μ-Hydroxysteroids as coenzymes of hydrogen transfer between di- and triphosphopyridine nucleotides, *J. Biol. Chem.*, 1958, **233,** 886.
10. Bielschowsky, F., Distant tumors produced by 2-amino- and 2-acetylaminofluorene, *Brit. J. Exp. Path.*, 1944, **25,** 1.
11. Edmonson, H. A., and Steiner, P. E., Primary carcinoma of the liver. A study of 100 cases among 48,900 necropsies, *Cancer*, 1954, **7,** 462.
12. Glock, G. E., and McLean, P., Further studies on the properties and assay of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase of rat liver, *Biochem. J.*, 1953, **55,** 400.
13. Glock, G. E., and McLean, P., Levels of enzymes of the direct oxidative pathway of carbohydrate metabolism in mammalian tissues and tumours, *Biochem. J.*, 1954, **56,** 171.
14. Glock, G. E., and McLean, P., A preliminary investigation of the hormonal control of the hexose monophosphate oxidative pathway, *Biochem. J.*, 1955, **61,** 390.
15. Rudolph, G. G., and Olsen, N. S., Effect of hypophysectomy on dehydrogenase activity of rat tissue, *Biochim. et Biophysica Acta*, 1956, **19,** 382.
16. Phillips, A. H., and Langdon, R. G., The influence of thyroxine and other hormones on hepatic TPN-cytochrome reductase activity, *Biochim. et Biophysica Acta*, 1956, **19,** 380.
17. Chatagner, F., and Bergeret, B. Influence de l'oestradiol sur la decarboxylation de l'acide cystéinesulfinique par la foie du rat adult ovariectomisé, *Compt. rend. Acad. Sc.*, 1957, **244,** 2422.
18. Bergeret, B., Labouesse, J., and Chatagner, F., Répercussion de la thyroidectomie et d'injections de thyroxine sur l'activité de quelques décarboxylases d'acids amines chez le rat, *Bull. Soc. chim. biol.*, 1958, **50,** 1923.
19. Brown, L. M., and Harrison, M. F., Effect of a single injection of carbon tetrachloride upon the activity of the pseudo-cholinesterase in the liver and serum of male and female rats, *Nature*, 1951, **168,** 83.
20. Fishman, W. H., and Farmelant, M. H., Effects of androgens and estrogens on β-glucuronidase in inbred mice, *Endocrinology*, 1953, **52,** 536.
21. Tadokoro, T., and Ito, K., Sex and enzyme action in the tissue, *J. Agric. Chem. Soc. Japan*, 1931, **7,** 171.

22. Sloane-Stanley, G. H., Amino-acid decarboxylases of rat liver, *Biochem. J.*, 1949, **45,** 556.
23. Talalay, P., Takano, G. M. V., and Huggins, C., Studies on the Walker tumor. II. Effects of adrenalectomy and hypophysectomy on tumor growth in tube-fed rats, *Cancer Research*, 1952, **12,** 838.
24. Kubowitz, F., and Ott, P., Isolierung und Kristallisation eines Garungsferments aus Tumoren, *Biochem. Z.*, 1943, **314,** 94.
25. King, E. J., and Armstrong, A. R., Convenient method for determining serum and bile phosphatase activity, *Canad. Med. Assn. J.*, 1934, **31,** 376.
26. Dawkins, M. J. R., Respiratory enzymes in the liver of the newborn rat, *Proc. Royal Soc. London, Series B*, 1959, **150,** 284.