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Effects of Hormones on Levels of Oxidized and Reduced Diphosphopyridine Nucleotide and Triphosphopyridine Nucleotide in Liver and Diaphragm

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(Received 5 April 1955)

Variations in tissue levels of diphosphopyridine nucleotide (DPN) and triphosphopyridine nucleotide (TPN), as well as the relative proportions of oxidized and reduced coenzymes, presumably play an important part in the regulation of alternative pathways of carbohydrate metabolism. For this reason, the methods described by Glock & McLean (1955*a*) for the separate determination of oxidized and reduced DPN and TPN in animal tissues have been applied to the estimation of DPN^+ , DPNH , TPN^+ and TPNH in the livers and voluntary muscles of rats subjected to various dietary and hormonal procedures. The present paper deals with the effects of starvation, alloxan diabetes and treatment with growth hormone, thyroxine and thiouracil. Changes in the total DPN content of liver, as well as in the proportion of oxidized to reduced coenzyme, have recently been reported by Helmreich, Holzer, Lamprecht & Goldschmidt (1954) in alloxan diabetes and in starvation.

METHODS

Treatment of animals. Young adult albino rats were used. The dietary procedure, production of alloxan diabetes, conditions for studying the effects of starvation and treatment with growth hormone, thyroxine and thiouracil were the same as described in the preceding paper (Glock & McLean, 1955*b*).

Estimation of coenzymes. The preparation of substrates and enzymes was carried out as described previously (Glock & McLean, 1955*a*), except for cytochrome *c*, which was a commercial preparation (Sigma Chemical Co.). Oxidized and reduced coenzymes were determined, respectively, in neutralized acid and alkaline tissue extracts (Glock & McLean, 1955*a*).

RESULTS

Alloxan diabetes and starvation

The contents of oxidized and reduced DPN and TPN in the liver and diaphragm of alloxan-diabetic and pair-fed control rats are given in Table 1. Liver values after 24 and 48 hr. starvation are shown in Table 2. In the case of the alloxan-diabetic rats, there was a significant fall in the concentration of oxidized DPN/g. liver with a corresponding decrease in the DPN^+/DPNH quotient. Although the total DPN ($\text{DPN}^+ + \text{DPNH}$) content was unaltered on a g. liver basis there was a significant decrease on a whole-liver basis. There were much less striking changes in the TPN contents. The total TPN (principally TPNH)/g. liver was unaffected, but again total TPN/whole liver was significantly reduced. In diaphragm there was some rise in the concentration of oxidized DPN/g. muscle, with a small but significant increase in the DPN^+/DPNH quotient, the total DPN concentration being unaffected. When expressed on a whole-diaphragm basis, there was a small but significant decrease in both DPNH and total DPN ($\text{DPN}^+ + \text{DPNH}$) contents. The total TPN content of diaphragm is too small to warrant further investigation.

The liver values of rats starved for 24 and 48 hr. (see Table 2) indicate that, as in alloxan diabetes, there is a marked fall in the DPN^+/DPNH quotient when compared with the average quotient of control rats (Table 1). In addition, there was a marked reduction in the concentration of both oxidized and reduced DPN when the starvation period was extended to 48 hr.

Growth-hormone treatment

Values for the DPN⁺, DPNH, TPN⁺ and TPNH contents of the livers of control and pair-fed growth-hormone-treated female rats are given in Table 3. As in previous experiments (Glock & McLean, 1955*b*), two different growth-hormone preparations were used, GH 1 and GH 2. Only

GH 1 produced any significant changes in co-enzyme levels, which can presumably be attributed to the fact that this preparation was more effective in promoting growth, particularly of the diaphragm, than GH 2. It is of interest that GH 1 was also much more effective than GH 2 in causing an increase in the G 6-P dehydrogenase activity of liver (Glock & McLean, 1955*b*).

Table 1. *Levels of oxidized and reduced coenzymes in liver and diaphragm of control and alloxan-diabetic rats*

Results expressed as means $\pm$ S.E.M. Duration of diabetes, 10 days.

	Control group	Alloxan-treated group	P
No. of animals	5	5	—
Initial body wt. (g.)	201 $\pm$ 5	201 $\pm$ 6	—
Final body wt. (g.)	230 $\pm$ 6	159 $\pm$ 10	—
Liver wt. (g.)	10.11 $\pm$ 0.48	7.33 $\pm$ 0.59	0.006
Diaphragm wt. (mg.)	380 $\pm$ 8	290 $\pm$ 17	0.001
Average daily sugar excretion (g.)	—	4.3 $\pm$ 0.5	—
Liver coenzymes (μ g./g.):			
DPN ⁺	407 $\pm$ 13	324 $\pm$ 18	0.007
DPNH	161 $\pm$ 17	217 $\pm$ 23	0.086
DPN ⁺ + DPNH	568 $\pm$ 27	541 $\pm$ 28	0.504
DPN ⁺ /DPNH	2.70 $\pm$ 0.31	1.53 $\pm$ 0.15	0.011
TPN ⁺	10 $\pm$ 1	8 $\pm$ 1	0.115
TPNH	148 $\pm$ 5	159 $\pm$ 6	0.195
Total liver coenzymes (μ g.):			
DPN ⁺	4140 $\pm$ 330	2387 $\pm$ 251	0.003
DPNH	1651 $\pm$ 247	1565 $\pm$ 129	0.772
DPN ⁺ + DPNH	5791 $\pm$ 541	3952 $\pm$ 315	0.019
TPN ⁺	104 $\pm$ 11	58 $\pm$ 10	0.017
TPNH	1496 $\pm$ 67	1153 $\pm$ 73	0.009
Diaphragm coenzymes (μ g./g.):			
DPN ⁺	263 $\pm$ 7	307 $\pm$ 17	0.050
DPNH	164 $\pm$ 25	143 $\pm$ 9	0.119
DPN ⁺ + DPNH	427 $\pm$ 12	450 $\pm$ 20	0.345
DPN ⁺ /DPNH	1.61 $\pm$ 0.08	2.18 $\pm$ 0.17	0.016
TPN ⁺	3 $\pm$ 1	2 $\pm$ 1	0.360
TPNH	12 $\pm$ 1	13 $\pm$ 2	0.847
Total diaphragm coenzymes (μ g.):			
DPN ⁺	100 $\pm$ 2	89 $\pm$ 6	0.132
DPNH	63 $\pm$ 3	42 $\pm$ 4	0.005
DPN ⁺ + DPNH	163 $\pm$ 5	131 $\pm$ 9	0.017

Table 2. *Effect of starvation on the levels of oxidized and reduced coenzymes in rat liver*

Results expressed as means $\pm$ S.E.M.

	Starved for 24 hr.	Starved for 48 hr.	P
No. of animals	6	6	—
Initial body wt. (g.)	182 $\pm$ 1	179 $\pm$ 2	—
Final body wt. (g.)	170 $\pm$ 3	153 $\pm$ 2	—
Liver wt. (g.)	5.50 $\pm$ 0.06	5.03 $\pm$ 0.17	0.026
Liver coenzymes (μ g./g.):			
DPN ⁺	333 $\pm$ 24	212 $\pm$ 26	0.008
DPNH	202 $\pm$ 16	121 $\pm$ 9	0.002
DPN ⁺ + DPNH	535 $\pm$ 35	333 $\pm$ 34	0.003
DPN ⁺ /DPNH	1.67 $\pm$ 0.12	1.74 $\pm$ 0.14	0.761
TPN ⁺	20 $\pm$ 6	< 2	—
TPNH	308 $\pm$ 34	308 $\pm$ 24	—
Total liver coenzymes (μ g.):			
DPN ⁺	1825 $\pm$ 118	1086 $\pm$ 184	0.007
DPNH	1111 $\pm$ 84	617 $\pm$ 66	0.001
DPN ⁺ + DPNH	2935 $\pm$ 175	1702 $\pm$ 246	0.002
TPN ⁺	109 $\pm$ 29	< 10	—
TPNH	1689 $\pm$ 184	1527 $\pm$ 88	0.648

As Table 3 shows, GH 1 produced a marked increase in DPN⁺, DPNH and total DPN (DPN⁺ + DPNH) contents/g. liver, all these increases being accentuated when expressed on a whole-liver basis. The concentration of TPNH/g. liver was not affected, but on account of the increase in liver size resulting from growth-hormone treatment, TPNH/whole liver was increased. No significant alterations were found in the coenzyme contents of diaphragm.

a whole-liver basis. The most marked effect was on the total TPN (i.e. principally TPNH) content of liver, which was strikingly decreased both on a unit-weight and on a whole-liver basis. No significant alterations in coenzyme concentrations were found in diaphragm.

Results for thiouracil-treated and pair-fed control rats are given in Table 5. Neither liver nor diaphragm showed any marked alterations in DPN or TPN contents.

Table 3. *Levels of oxidized and reduced coenzymes in liver and diaphragm of control and growth-hormone-treated rats*

Results expressed as means $\pm$ S.E.M.

	Control group	Growth-hormone-treated group*	P	Control group	Growth-hormone-treated group†	P
No. of animals	5	5	—	11	11	—
Initial body wt. (g.)	158 $\pm$ 4	157 $\pm$ 4	—	186 $\pm$ 6	184 $\pm$ 5	—
Final body wt. (g.)	164 $\pm$ 7	180 $\pm$ 4	—	192 $\pm$ 5	205 $\pm$ 4	—
Liver wt. (g.)	5.96 $\pm$ 0.51	7.50 $\pm$ 0.81	0.148	6.53 $\pm$ 0.27	7.16 $\pm$ 0.26	0.105
Diaphragm wt. (mg.)	403 $\pm$ 15	492 $\pm$ 27	0.023	366 $\pm$ 22	394 $\pm$ 22	0.379
Liver coenzymes (μ g./g.):						
DPN ⁺	330 $\pm$ 9	421 $\pm$ 38	0.051	289 $\pm$ 17	326 $\pm$ 10	0.069
DPNH	133 $\pm$ 8	206 $\pm$ 27	0.035	136 $\pm$ 12	118 $\pm$ 9	0.264
DPN ⁺ + DPNH	463 $\pm$ 17	627 $\pm$ 49	0.014	425 $\pm$ 20	444 $\pm$ 16	0.472
DPN ⁺ /DPNH	2.49 $\pm$ 0.09	2.24 $\pm$ 0.43	0.565	2.30 $\pm$ 0.22	2.91 $\pm$ 0.22	0.068
TPN ⁺	10 $\pm$ 1	6 $\pm$ 2	0.059	3 $\pm$ 1	2	—
TPNH	185 $\pm$ 16	199 $\pm$ 18	0.565	193 $\pm$ 10	196 $\pm$ 9	0.828
Total liver coenzymes (μ g.):						
DPN ⁺	1962 $\pm$ 181	3157 $\pm$ 453	0.040	1897 $\pm$ 147	2332 $\pm$ 110	0.028
DPNH	788 $\pm$ 69	1465 $\pm$ 111	0.001	871 $\pm$ 64	845 $\pm$ 71	0.761
DPN ⁺ + DPNH	2749 $\pm$ 247	4622 $\pm$ 427	0.005	2767 $\pm$ 157	3164 $\pm$ 167	0.099
TPN ⁺	56 $\pm$ 7	38 $\pm$ 10	0.172	19 $\pm$ 6	<14	—
TPNH	1104 $\pm$ 142	1449 $\pm$ 91	0.076	1268 $\pm$ 96	1403 $\pm$ 81	0.284
Diaphragm coenzymes (μ g./g.):						
DPN ⁺	383‡	366‡	—	257 $\pm$ 16	252 $\pm$ 15	0.822
DPNH	271	236	—	97 $\pm$ 16	105 $\pm$ 19	0.755
DPN ⁺ + DPNH	656	602	—	354 $\pm$ 25	357 $\pm$ 16	0.922
DPN ⁺ /DPNH	1.42	1.55	—	2.96 $\pm$ 0.35	2.88 $\pm$ 0.44	0.890
TPN ⁺	3	3	—	<2	<2	—
TPNH	18	15	—	14 $\pm$ 1	11 $\pm$ 3	0.608

* Female hooded Norway rats injected daily subcutaneously with 400 μ g. growth-hormone preparation 1 for 8 days.

† Female albino rats injected daily subcutaneously with 500 μ g. growth-hormone preparation 2 for 10 days. (For description of growth-hormone preparations see Glock & McLean, 1955b.)

‡ Values for pooled diaphragm extracts.

The total liver-DPN values in this experiment were considerably lower than in any of the others. This may, perhaps, be attributed to the fact that this was the only experiment employing female rats.

Thyroxine and thiouracil treatment

The coenzyme contents of the liver and diaphragm of control and pair-fed thyroxine-treated rats are given in Table 4. Thyroxine treatment resulted in a fall in the concentration of oxidized DPN/g. liver and a marked decrease in both oxidized DPN and total DPN (DPN⁺ + DPNH) on

DISCUSSION

Both starvation and experimental diabetes result in a marked inhibition of fatty acid synthesis. In the former case, this can be restored to normal by glucose only, whereas both glucose and insulin are necessary to reverse the diabetic condition (see Chaikoff, 1951–52; Stadie, 1954). Lipogenesis is dependent on the supply of acetyl coenzyme A and DPNH, derived from the breakdown of carbohydrate. For lipogenesis to proceed at a significant rate, it is necessary to maintain a high concentration of both these substrates in order to shift the

Table 4. *Levels of oxidized and reduced coenzymes in liver and diaphragm of control and thyroxine-treated rats*Results expressed as means $\pm$ S.E.M.

	Control group	Thyroxine-treated group	P
No. of animals	6	6	—
Initial body wt. (g.)	170 $\pm$ 1	172 $\pm$ 2	—
Final body wt. (g.)	207 $\pm$ 3	170 $\pm$ 4	—
Liver wt. (g.)	8.57 $\pm$ 0.09	7.26 $\pm$ 0.21	<0.001
Diaphragm wt. (mg.)	350 $\pm$ 13	288 $\pm$ 9	0.003
Liver coenzymes (μ g./g.):			
DPN ⁺	446 $\pm$ 23	393 $\pm$ 7	0.057
DPNH	158 $\pm$ 9	164 $\pm$ 10	0.628
DPN ⁺ + DPNH	604 $\pm$ 22	557 $\pm$ 16	0.113
DPN ⁺ /DPNH	2.86 $\pm$ 0.25	2.43 $\pm$ 0.12	0.150
TPN ⁺	4 $\pm$ 1	<2	—
TPNH	206 $\pm$ 8	155 $\pm$ 10	0.003
Total liver coenzymes (μ g.):			
DPN ⁺	3818 $\pm$ 195	2856 $\pm$ 111	0.001
DPNH	1357 $\pm$ 81	1193 $\pm$ 85	0.192
DPN ⁺ + DPNH	5176 $\pm$ 188	4049 $\pm$ 185	0.001
TPN ⁺	30 $\pm$ 10	<14	—
TPNH	1762 $\pm$ 73	1119 $\pm$ 68	<0.001
Diaphragm coenzymes (μ g./g.):			
DPN ⁺	301 $\pm$ 26	354 $\pm$ 25	0.172
DPNH	109 $\pm$ 13	102 $\pm$ 10	0.631
DPN ⁺ + DPNH	424 $\pm$ 27	456 $\pm$ 33	0.504
DPN ⁺ /DPNH	3.06 $\pm$ 0.57	3.57 $\pm$ 0.22	0.347
TPN ⁺ (pooled values)	<2	<2	—
TPNH (pooled values)	14	15	—
Total diaphragm coenzymes (μ g.):			
DPN ⁺	104 $\pm$ 8	102 $\pm$ 7	0.845
DPNH	36 $\pm$ 4	29 $\pm$ 3	0.144
DPN ⁺ + DPNH	142 $\pm$ 4	131 $\pm$ 9	0.347

Table 5. *Levels of oxidized and reduced coenzymes in liver and diaphragm of control and thiouracil-treated rats*Results expressed as means $\pm$ S.E.M.

	Control group	Thiouracil-treated group	P
No. of animals	6	6	—
Initial body wt. (g.)	163 $\pm$ 3	163 $\pm$ 2	—
Final body wt. (g.)	233 $\pm$ 5	206 $\pm$ 4	—
Liver wt. (g.)	8.27 $\pm$ 0.22	9.04 $\pm$ 0.23	0.037
Diaphragm wt. (mg.)	403 $\pm$ 20	377 $\pm$ 12	0.297
Liver coenzymes (μ g./g.):			
DPN ⁺	370 $\pm$ 19	363 $\pm$ 13	0.770
DPNH	204 $\pm$ 11	211 $\pm$ 17	0.698
DPN ⁺ + DPNH	574 $\pm$ 29	574 $\pm$ 27	—
DPN ⁺ /DPNH	1.82 $\pm$ 0.07	1.75 $\pm$ 0.10	0.562
TPN ⁺	8 $\pm$ 3	14 $\pm$ 2	0.054
TPNH	309 $\pm$ 22	311 $\pm$ 16	0.922
Total liver coenzymes (μ g.):			
DPN ⁺	3044 $\pm$ 118	3292 $\pm$ 190	0.297
DPNH	1680 $\pm$ 77	1917 $\pm$ 174	0.244
DPN ⁺ + DPNH	4724 $\pm$ 179	5209 $\pm$ 340	0.236
TPN ⁺	63 $\pm$ 8	128 $\pm$ 5	0.003
TPNH	2546 $\pm$ 164	2809 $\pm$ 145	0.273
Diaphragm coenzymes (μ g./g.):			
DPN ⁺	217 $\pm$ 11	192 $\pm$ 11	0.141
DPNH	103 $\pm$ 3	100 $\pm$ 5	0.341
DPN ⁺ + DPNH	319 $\pm$ 14	302 $\pm$ 14	0.389
DPN ⁺ /DPNH	2.11 $\pm$ 0.07	1.74 $\pm$ 0.10	0.012
TPN ⁺ (pooled values)	2	10	—
TPNH (pooled values)	13	40	—
Total diaphragm coenzymes (μ g.):			
DPN ⁺	87 $\pm$ 4	72 $\pm$ 4	0.023
DPNH	41 $\pm$ 2	41 $\pm$ 1	—
DPN ⁺ + DPNH	128 $\pm$ 5	113 $\pm$ 5	0.048

equilibrium of β -ketothiolase and β -ketoreductase in the direction of synthesis (see Lynen, 1954; Green, 1954). In diabetes and starvation, where the metabolism of carbohydrate is depressed and the synthesis of fatty acids restricted, an alteration in the steady-state level of DPN^+/DPNH would be expected. A change in the DPN^+/DPNH quotient has indeed been found in rat livers in starvation and diabetes both by Helmreich *et al.* (1954) and by us. The former workers obtained an increase in the DPN^+/DPNH quotient due chiefly, but not entirely, to a decrease in the concentration of DPNH , whereas we have found a decreased DPN^+/DPNH quotient resulting from a fall in the concentration of DPN^+ . It is difficult to account for these diametrically opposed results. The only obvious differences in experimental procedures are that Helmreich *et al.* (1954) did not employ the paired-feeding technique, the effects of starvation were studied after as long as 72–120 hr., when they were no longer reversible by glucose, and the degree of diabetes was probably more severe since several rats were killed in diabetic coma. These workers consider that ketone-body accumulation and decreased DPNH concentrations in starvation and diabetes are the outcome of deranged glycolysis and that in both conditions low concentrations of DPNH are responsible for restricted fatty acid synthesis. Our results, on the other hand, could be explained on the basis that lipogenesis is known to be more strikingly reduced than the glycolytic breakdown of glucose in both diabetic and fasting rats (see Chaikoff, 1951–52; Stadie, 1954) and that fatty acid oxidation proceeds normally in diabetic liver (see Green, 1954). Thus two of the enzyme systems causing reduction of DPN^+ to DPNH are relatively unimpaired, whereas lipogenesis, a synthetic process requiring DPNH , is strongly inhibited. This would result in an accumulation of DPNH and a reduction in the level of oxidized DPN . It is of interest that the DPN^+/DPNH quotient is unchanged in the livers of rats after growth-hormone treatment at a time when there is increased fat catabolism, as shown by changes in R.Q. and body composition (Greenbaum, 1953; Young, 1953).

It is now generally believed that the action of thyroxine in controlling tissue respiration is located not at the level of the individual respiratory enzymes but in the mitochondria, where respiration is coupled to the production of high-energy phosphate bonds. Under specified conditions, both thyroxine and triiodothyronine can uncouple oxidative phosphorylation in mitochondrial preparations *in vitro* (see Maley & Lardy, 1953; Lardy & Maley, 1954) and the *in vitro* incorporation of ^{32}P into mitochondrial ATP has also been shown to be depressed (Martius & Hess, 1951). Moreover,

reduced P:O ratios have been reported in mitochondrial preparations from hyperthyroid animals by two groups of investigators (Lardy & Feldott, 1951; Hoch & Lipmann, 1953). It should be emphasized, however, that these effects can be demonstrated only under certain specified conditions using large unphysiological doses of thyroxine (see Du Toit, 1952). Reduced mitochondrial synthesis of ATP in hyperthyroidism could account for the reduced DPN and TPN concentration in the livers of rats after thyroxine treatment. The more or less uniform intracellular distribution of $[^{131}\text{I}]$ -thyroxine between mitochondrial, nuclear and supernatant liver fractions (Lipner, Klitgaard, Barker & Winnick, 1952; Crane, 1952) would favour any active participation of this hormone in the mitochondrial synthesis of ATP and in nuclear coenzyme synthesis. An effect of thyroxine on coenzyme synthesis is, however, purely hypothetical. It is of interest that whereas thyroxine results in a marked reduction in total DPN and TPN concentrations in liver, diaphragm coenzyme levels are not appreciably affected. This can perhaps be attributed to the fact that injected $[^{131}\text{I}]$ thyroxine is preferentially concentrated in the liver (Albert & Keating, 1952).

Marked variations in total DPN and TPN tissue concentrations, such as occur in rat livers as a result of thyroxine and growth-hormone treatments, are presumably due to direct or indirect effects of these hormones on coenzyme synthesis. It would be interesting to see whether hormones have any *in vitro* effects on coenzyme synthesis in isolated nuclei.

SUMMARY

1. Oxidized and reduced DPN and TPN contents of liver and diaphragm have been determined in control, diabetic and fasting rats and in rats subjected to treatment with growth hormone, thyroxine and thiouracil.
2. Total DPN ($\text{DPN}^+ + \text{DPNH}$) contents of liver were markedly reduced in alloxan diabetes and after 48 hr. starvation. A decrease was also found after thyroxine treatment. Striking increases in total DPN contents of liver were, on the contrary, found after treatment with one crystalline growth-hormone preparation but not with a less active preparation.
3. Total liver TPN (principally TPNH) contents were strikingly reduced as a result of thyroxine treatment, although thiouracil produced no significant changes.
4. The DPN^+/DPNH quotient of rat liver was significantly reduced in alloxan diabetes.
5. Alterations in diaphragm coenzyme contents were less striking than those found in liver.

6. An attempt has been made to account for the low DPN⁺/DPNH quotient found in diabetic liver, and it has been tentatively suggested that hormones may directly or indirectly regulate the synthesis of both DPN and TPN.

This work was carried out during the tenure of the Hugh Percy Noble Scholarship by G.E.G. and has been in part financed by a grant from the Research Fund of the University of London and also by a grant to the Medical School from the British Empire Cancer Campaign. We are indebted to Professor F. Dickens, F.R.S., for his interest and to Miss Audrey Bowles for valuable technical assistance.

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The Metabolism of Phospholipids in the Lactating Mammary Gland of the Rabbit

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(Received 17 March 1955)

The experiments to be described were undertaken as part of a study to determine the role of blood lipids in the metabolism of the lactating mammary gland. Contrary to the views formerly held (e.g. Meigs, Blatherwick & Cary, 1919) that plasma phospholipids are the precursors of the fatty acids of milk fat, arteriovenous studies subsequently indicated that no uptake of phospholipids into the lactating bovine gland apparently takes place (Blackwood, 1934) and that no fraction of blood lipids except glycerides and possibly cholesteryl esters are absorbed from the blood stream by the ruminant udder (Lintzel, 1934; Graham, Jones & Kay, 1936; Maynard, McCay, Ellis, Hodson & Davis, 1938; Shaw & Petersen, 1940; Voris, Ellis & Maynard, 1940). Aten & Hevesy (1938), in one of the first studies of lactation involving radioisotopes, showed that phospholipids are synthesized in the lactating udder of the goat, but contended that their results were not consistent with the view that plasma phospholipids are degraded in the mammary gland.

It will be shown, however, that in the lactating rabbit, phospholipids are not only synthesized in the mammary gland, but are also absorbed from the plasma and degraded in the gland to a significant extent. Though this study was primarily

concerned with the metabolism of lactating mammary gland tissue, the opportunity was taken to examine the corresponding phosphorus-containing fractions of the livers of the animals under investigation. A preliminary report of part of this work has already appeared (Garton & Popják, 1952).

EXPERIMENTAL

Administration of ³²P-labelled inorganic phosphate to lactating rabbits

Two lactating Chinchilla rabbits were injected intravenously with 57.5 μ C (rabbit 1) and 66 μ C (rabbit 2) of [³²P]orthophosphate contained in 0.9% NaCl. Two hours later rabbit 1 was anaesthetized with nembutal-ether and mammary-gland tissue removed, together with a piece of liver. The tissue samples were carefully blotted with filter paper, cut into small pieces and preserved in a freezing mixture of acetone and solid CO₂ below -70°. Rabbit 2 was similarly treated, except that before tissue samples were taken the animal was given a subcutaneous injection of about 2 i.u. oxytocin (Pitocin, Parke, Davis and Co. Ltd., Hounslow, Middlesex) and milked, the suction technique described by Popják, Hunter & French (1953) being used to yield about 20 ml. milk; in addition, samples of mammary gland and liver were taken and blood was withdrawn from the aorta into heparinized receivers and immediately centrifuged to obtain plasma.