Our experiments suggest that when the blood sedimentation rate is raised, there is no alteration in the chemical structure of the proteins. A possible exception to this is the Bence-Jones protein [Devine, 1941]. Geottsch [1936] found that nephritic total globulin was immunologically different from the normal, and Alving [1936] found changes in the cystine content of total albumin in nephrosis. Hewitt [1938*b*] later showed that in both cases the individual fractions of globulin or albumin give normal chemical and immunological reactions, and the altered ratios are responsible for the abnormality of the plasma.

SUMMARY

1. The proteins from normal plasma were separated into nine fractions, and the rate of fall of a

20% vol. of erythrocytes in 3% solutions of the proteins during 1 hr. was found to range from 100 mm. for fibrinogen to 1.5 mm. for total albumin.

2. The fractions were combined in equal amounts using 39 pairs. The action of the 'faster' fractions, fibrinogen and euglobulin, is inhibited by the 'slow' nucleoprotein and globoglycoid.

3. Artificial pathological plasmas built up from these fractions gave sedimentation rates similar to those for the comparable natural material. With artificial tuberculous plasma it was found that by altering only the component globulin fractions, the sedimentation rate could be changed from 94 to 26 mm.; and with artificial nephritic blood, substitution of total albumin by crystalalbumin altered the rate from 15.0 to 145 mm., while substitution of the total albumin by globoglycoid changed it from 15.0 to 1.0 mm.

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Phosphorolysis and Synthesis of Glycogen in Animal Tissues

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The mechanism of glycogenolysis in muscle and liver is described by Cori, Cori & Schmidt [1939] as follows:

- (1) Glycogen + phosphate $\rightleftharpoons$ glucose-1-phosphate (catalysed by phosphorylase and adenylic acid).
- (2) Glucose-1-phosphate $\rightleftharpoons$ hexose-6-phosphate (catalysed by phosphoglucomutase and Mg^{++}).
- (3) Hexose-6-phosphate $\rightleftharpoons$ glucose + phosphate (catalysed by phosphatase in liver).
- (3*a*) Hexose-6-phosphate $\rightleftharpoons$ hexose-1:6-diphosphate $\rightarrow$ lactic acid (in muscle).

These reactions are responsible for glucose formation in liver, and are the first steps in glycolysis in muscle. Glycogen resynthesis too is probably explained by the same reactions [Cori & Cori, 1941]. It seemed of interest, therefore, to study the distri-

bution of the enzymes concerned, in various tissues at various stages of development, and to compare the enzymic activities with the rate of metabolism of glycogen in the tissue concerned. The further question was also investigated in this connexion whether insufficiency of one or more of the designated enzymes is the cause of metabolic disturbances in certain pathological conditions.

A separate series of experiments dealt with some properties of phosphorylase, and in particular with its inhibition by glucose. This inhibition was first observed by Lehmann [1938] who suggested its possible physiological importance. It may be responsible for the homeostatic mechanism described by Soskin, Essex, Herrick & Mann [1938].

METHODS

Preparation of phosphorylase

Phosphorylase was prepared according to Cori *et al.* [1939] by adsorbing the enzyme of tissue extracts on $\text{Al}(\text{OH})_3$ and eluting with Na glycerophosphate. It was found, however, that $\text{C}_\alpha \text{Al}(\text{OH})_3$ prepared according to Willstätter, Kraut & Erbacher [1925] frequently yielded inactive enzyme preparations. With the C_β variation, on the other hand, active preparations were regularly obtained, and remained active for long periods provided the precipitate had been washed only once with NH_4OH instead of twice.

Estimation of enzymic activity

(a) *Phosphorylase activity* was estimated on the basis of the amount of glycogen synthesized from glucose-1-phosphate in the presence of the enzyme. A mixture of 1 vol. enzyme solution and $\frac{1}{2}$ vol. of a solution containing 3% glucose-1-phosphate and 1½% glycogen in $N/10 \text{ H}_2\text{SO}_4$ is incubated at 25° for various periods. In some cases 0.25 mg. adenylic acid is added per ml. of the mixture. The extent of glycogen synthesis was determined by measuring the amount of inorganic phosphate liberated.

(b) *Phosphoglucumutase activity* was estimated as the decrease in easily hydrolysable phosphate (10 min. hydrolysis with $N/1 \text{ HCl}$ at 100°) when glucose-1-phosphate is added to 0.3 g. of tissue brei suspended in 2 ml. of bicarbonate Ringer containing $M/40 \text{ NaF}$, and incubated at 37° with continuous shaking. The bicarbonate Ringer is composed of 4 ml. 1.15% KCl , 1 ml. 0.8% MgSO_4 , 100 ml. 0.9% NaCl and 60 ml. 1.3% NaHCO_3 . Before use the solution is saturated with CO_2 .

(c) *Glycogen phosphorylase*. The conversion of glycogen into hexose-6-phosphate was measured by estimating the disappearance of inorganic phosphate when 0.3 g. of tissue brei was incubated with 1% glycogen and $M/40 \text{ NaF}$ in phosphate-Ringer at 37° with continuous shaking. The phosphate-Ringer is similar in composition to the bicarbonate-Ringer except that phosphate buffer $M/10$ of pH 7.4 is used instead of bicarbonate.

(d) *Estimation of phosphate*. Phosphate was estimated according to Lohmann & Jendrasik [1926] after the reactions had been stopped by the addition of 1 ml. 20% CCl_3COOH . In the case of glycogen synthesis from glucose-1-phosphate the acid solution is immediately diluted to avoid splitting of the ester by acid.

RESULTS

Distribution of the phosphorolytic system

Phosphorylase was found in almost all tissues (Fig. 1). Organs with a highly active glycogen metabolism, such as muscle, liver and heart, however, give much more active phosphorylase preparations than organs which are devoid of, or have only a slight glycogen metabolism, as, for example, lungs, skin, testes, intestine, spleen, adipose tissue and placenta. Kidney and brain occupy an intermediary position as regards both phosphorylase activity and glycogen metabolism.

The distribution of phosphoglucumutase and glycogen phosphorylase is generally parallel to the

Fig. 1. Glycogen synthesis from glucose-1-phosphate by phosphorylase in tissue brei. *a*, muscle; *b*, heart and subcutis; *c*, liver; *d*, kidney; *e*, brain; *f*, tumour; *g*, lung and intestine; *h*, skin, testes and spleen; *i*, adipose; *k*, placenta.

Fig. 2. Conversion of glucose-1-phosphate into hexose-6-phosphate in tissue brei. *a*, muscle; *b*, heart; *c*, skin; *d*, brain; *e*, spleen; *f*, lung; *g*, tumour; *h*, intestine, adipose and testes.

Fig. 3. Phosphorolysis of glycogen in tissue brei. *a*, muscle; *b*, liver; *c*, heart; *d*, brain; *e*, kidney; *f*, skin; *g*, lung; *h*, tumour; *i*, intestine, testes, adipose, pancreas and placenta.

intensity of glycogen metabolism. Yet several exceptions were observed (Fig. 2, 3). Tumour tissue (sarcoma), though it is not generally classed as a glycogen-metabolizing tissue, displayed a high phosphorylase activity. The phosphorolytic breakdown of glycogen by this tissue is, however, very slow as compared with that of other tissues. This apparent discrepancy is due to the low phosphoglucomutase activity of tumour tissue.

Glycogen metabolism of subcutaneous tissue

A further apparent disagreement occurs with subcutaneous tissue. This tissue is one of the most active as regards the phosphorylase activity, but was not previously known to possess any high glycogen-metabolizing capacity. It was found, however, that this tissue has a regular and high glycogen metabolism (Table 1), and that subcutaneous tissue from normal rats contains quantities

Table 1. *Glycogen content of subcutaneous tissues*

Treatment of animal	No. of exp.	Blood sugar (mg./100 ml.)	Average glycogen content of subcutaneous tissues %
Normal rats	24	121	0.27
1-2 days' fast	12	99	0.06
0.03 mg. adrenaline 1-2 hr. previously	5	175	0.06
0.005-0.01 mg. adrenaline 1-2 hr. previously	5	125	0.05
6 ml. 25% glucose <i>per os</i> , in addition to regular diet	8	110	0.50

of glycogen comparable with those of muscle tissue. This glycogen store is readily mobilized during fasting and thus becomes depleted. On administration of adrenalin the glycogen content drops. This decrease was observed with doses of adrenalin causing an elevation of blood sugar, as well as with low doses having no marked effect on the blood-sugar level. Sugar administration by stomach tube caused a marked elevation of the glycogen content of the subcutaneous tissues.

When rats received a normal, carbohydrate-rich diet after prolonged undernutrition (causing 20% loss of body wt.), an exceptionally high glycogen

content was found in the subcutaneous tissues on the 1st day after recommencement of the normal feeding. After 7 days on the usual carbohydrate-rich diet the glycogen content is again normal. In Table 2 the results with subcutaneous tissue are compared with those for glycogen in adipose tissue and in liver, under similar conditions [Tuerkischer & Wertheimer, 1942].

Subcutaneous tissue may thus be regarded as a glycogen-metabolizing tissue, and appears to constitute a supplementary glycogen store for the body.

The development of the phosphorolytic system

It is known, largely from the work of Needham and his collaborators [Needham & Lehmann, 1937; Needham & Nowinski, 1937], that embryonic tissue acts preferably on glucose, and only very slowly on glycogen, to form lactic acid. Chick embryo is rarely able to phosphorylate glycogen. The glycogenolytic system in various organs develops only with advancing age. Experiments were undertaken in order to identify the rate-limiting component of the glycogenolytic system, and to clarify the course of its development.

In muscle tissue from rats up to 10 days of age no phosphorylase activity could be detected (Table 3). This lack of activity is not due to the lack of the coenzyme adenylic acid, since even after addition of optimal amounts of coenzyme only an insignificant glycogen synthesis took place. As Table 3 shows, muscle phosphorylase activity rises rapidly after the 10th day and is substantial by the 14th day. At this stage the amount of adenylic acid present in muscle is the limiting factor, since the addition of this substance caused an acceleration of glycogen synthesis which is never observed in adult muscle tissue.

Table 3. *Glycogen synthesis in muscle tissue from young rats*

Age in days	Glucose-1-phosphate converted into glycogen in 1 hr. (% theoretical)	
	Without addition of adenylic acid %	With addition of adenylic acid %
10	0	5
14	26	45
20	25	52

Table 2. *Glycogen content of subcutaneous tissues after normal feeding of rats previously subjected to prolonged undernutrition*

Tissue	After 7 days' undernutrition wt. loss = 20 %	Glycogen content of tissue (%)						
		Days after re-feeding with carbohydrate-rich diet						
Subcutaneous tissue	0.07	1	2	3	4	5	6	7
'White' adipose tissue	0.00	0.58	0.92	0.61	0.51	0.50	0.55	0.32
'Brown' adipose tissue	0.00	0.55	0.90	0.50	0.23	Trace	0.00	0.00
Liver	0.00	1.85	2.40	1.80	0.27	Trace	0.00	0.00
	0.40	7.2	6.3	6.0	3.5	3.1	—	—

The rate of development of the glycogenolytic system in brain is quite different from that in muscle. The activity of the various components of the phosphorolytic system in relation to the activity of normal adult tissue—taking the last as 100—is shown in Fig. 4. It can be seen that in contrast to

Fig. 4. Phosphorolysis, glycogen synthesis, and phosphoglucumutase action in brain of rats at different ages. *a*, glycogen synthesis; *b*, phosphorolysis; *c*, phosphoglucumutase.

the results with muscle, the glycogen-synthesizing capacity is already present immediately after birth to the extent of about 50% of the adult value. Nevertheless, no phosphorolytic breakdown of glycogen takes place at this age. This is accounted for by the complete lack of phosphoglucumutase activity. Thus, the glucumutase and not the phosphorylase is the limiting factor in glycogenolysis in the brain of young rats. As Fig. 4 shows, the phosphorolytic capacity of brain parallels the development of the mutase activity. Following an initial period of a very slow development up to the age of 10 days, the mutase begins to develop quickly, and at 14 days about 70% of the adult activity is reached. After this age the results become less uniform. In general, the adult value for both mutase and glycogen phosphorolysis is reached at an age between 20 and 30 days. The phosphorylase system develops independently. Its relative activity was always higher than other systems examined. Hence, it can at no stage be regarded as the limiting factor of glycogen metabolism during development.

It remained to be established whether the development of the enzymic activities is due to the development of new kinds of cells or is the result of formation of new enzymes in cell types already present at birth. For this purpose the activity of white and of grey brain matter was compared. The results (Table 4) show that no significant difference in glycogen phosphorolysis can be observed in white and grey matter of brain. The development of the phosphorylase system thus seems to take place in all parts of the brain and not be specially due to the development of the grey matter.

Table 4. *Phosphorolysis of glycogen in white and grey matter of rabbit brain*

Tissue	Hexose phosphate formed (mg. P_2O_5) in		
	30 min.	60 min.	120 min.
(1) White matter	0.50	0.85	1.00
Grey matter	0.48	0.65	0.95
(2) White matter	0.60	0.85	0.98
Grey matter	0.64	0.93	1.10
Nerve fibre	0.48	0.63	0.70

Phosphorolysis in pathological conditions

Since it had been shown that a parallelism exists between the capacity of an organ to metabolize glycogen and the activity of its phosphorolytic system, it was of interest to determine whether a deficiency in the glycogen phosphorolytic system could be found in pathological cases in which glycogen metabolism is disturbed.

The effect of liver damage produced by various methods was first examined.

Table 5. *Phosphorolysis of glycogen in animals subjected to liver damage*

Animal	Experi- mental treat- ment	Initial glyco- gen con- tent %	Fat con- tent %	Hexose phos- phate formed in brei (mg. P ₂ O ₅) during		
				15 min.	30 min.	60 min.
(a) Liver tissue:						
Guinea-pig	None	2.4	4	1.32	2.05	2.45
"	CCl ₄	0.4	8	0.70	1.45	1.60
"	"	0.3	9	0.70	1.43	1.63
"	"	0.0	8	0.48	0.87	1.23
"	"	0.0	9	0.42	0.78	1.10
"	P	2.0	5	0.95	1.50	2.25
"	"	Trace	8	0.62	1.24	1.82
"	"	"	11	0.48	0.55	0.86
"	"	"	10	0.54	0.68	0.94
Rabbit	None	2.0	4	1.10	1.82	2.32
"	CCl ₄	0	7	0.30	0.77	0.93
"	"	0	9	0.00	0.00	0.40
"	CHCl ₃	0.4	9	0.25	0.53	0.85
"	"	0.5	8	0.37	0.64	0.98
"	Phlor- rhizin (fasting)	0.0	8	0.82	1.36	1.67
"	"	0.2	9	0.75	1.24	1.75
(b) Muscle tissue:						
Guinea-pig	None.	—	—	1.45	2.03	2.40
"	CCl ₄	—	—	1.67	2.50	2.55
"	"	—	—	1.33	2.05	2.18
"	"	0	8	1.45	2.10	2.40
"	"	0	9	1.30	2.08	2.43
"	P	2.0	5	1.80	2.60	2.80
"	"	Traces	8	2.44	2.74	2.94
"	"	"	11	1.68	2.30	2.56
"	"	"	10	1.63	2.00	2.40

It is evident from the data (Table 5) that livers damaged by poisons such as CCl_4 , P and CHCl_3 show impaired glycogen phosphorolysis breakdown. The fat and glycogen contents of liver served as measures of liver damage. It may be seen that when the liver fat has been raised above 8%, with low glycogen content, by the administration of the above poisons, the phosphorolytic capacity of the liver is far below normal. When fat accumulation was induced in the liver without damage, by prolonged fasting and phlorrhizin treatment, no comparable loss of phosphorolytic capacity was observed.

The diminution in phosphorolytic activity does not seem to be due to inactivation of phosphorylase, since, even in cases of almost complete lack of phosphorolytic activity, phosphorylase preparations from the same liver synthesize glycogen normally. The diminution may be due to an inactivation of the phosphoglucomutase. Further proof of this assumption is not available, since the estimation of phosphoglucomutase in liver is not practicable, liver phosphatase being active even in the presence of high concentrations of fluoride. In liver brei glucose-1-phosphate is split to give inorganic phosphate without the accumulation of hexose-6-phosphate.

Phosphorolysis in muscle tissue from adrenalectomized and thyroidectomized rats

Phosphorolysis was examined in muscles of adrenalectomized rats maintained in good condition by Rubin-Kriek's salt solution. Rats were used 10–30 days after adrenalectomy, and estimations were carried out after 10–30 min. incubation of the brei. Phosphorolysis in muscle of such rats was either normal or slightly diminished. The results agree with those of Holmes & Lehmann [1940] who, working on liver, found no loss of phosphorolysis activity in minced liver of adrenalectomized rabbits. They contradict those of Verzar & Montigel [1942], who found that phosphorolysis in the muscles of adrenalectomized rats was almost completely abolished when short incubation times (up to 30 min.) were used. It has, however, been shown by Holmes & Lehmann [1940] that the glycogen synthesis from glucose by rabbit-liver slices is lowered when the animals used were adrenalectomized. Bendall & Lehmann [1942] obtained the same result with rat-liver slices; they could restore the normal activity by a previous injection with extract from the suprarenal cortex.

Thyroidectomized rats also showed normal phosphorolytic power. The rats were maintained before and after operation on a diet containing fresh milk. Similar results were obtained throughout an interval of 1–4 months after operation.

In thyrotoxicosis also, phosphorolytic activity was found to be normal.

Properties of the phosphorylase system

(a) *Heat inactivation.* The phosphorylase of muscle of rats and rabbits is highly thermolabile. Heating to 60° for 5 min. causes complete destruction of the enzyme. At 55° , 20 min. were needed for complete destruction, whereas at 50° even after 60 min. some activity was preserved.

(b) *Ultra-violet irradiation.* Phosphorylase from rabbit muscle was irradiated by a mercury lamp at a distance of 50 cm. for 30 min. The temperature was not allowed to rise above 30° . Controls were maintained under the same conditions but covered to prevent penetration of ultra-violet light. The irradiation caused partial or complete inactivation of phosphorylase activity.

(c) *Inhibition by glucose.* In confirmation of the results of Lehmann [1938], Gill & Lehmann [1939], Lehmann [1939] and of Cori & Cori [1940], glycogen phosphorylation was found to be inhibited by glucose. A concentration of glucose of about 2% caused almost complete inhibition. On the other hand, in contradiction to Cori's results, glucose inhibition was found to be counteracted by adenylic acid, as is shown in Tables 6 and 7.

Table 6. *Inhibition by glucose of phosphorolysis in incubated muscle brei; influence of adenylic acid in counteracting this inhibitory action of glucose*

Material added	Hexose phosphate formed in 15 min. mg. P_2O_5	Observed inhibition of hexose phosphate formation (% of max. possible)
None	2.13	0
Glucose (initial conc. = 2%)	0.35	84
Adenylic acid (3 mg./ml.)	1.97	0
Adenylic acid (3 mg./ml.) plus glucose (initial conc. = 2%)	1.35	30

Na lactate, as well as Na pyruvate, caused only partial inhibition of glycogen synthesis even at concentrations as high as 2%. Adenosine-5-phosphoric acid (referred to here as adenylic acid) cannot be replaced by adenosine-3-phosphoric acid as a counteractant of glucose inhibition.

The discrepancy between these results and those of Cori is explained by the fact that Cori used lower concentrations of glucose. As is shown in Table 7, when concentrations of 0.3% glucose are used no influence of adenylic acid is detected. Addition of adenylic acid causes the reactivation of the enzyme only to a certain degree, so that after addition of adenylic acid the same activity is found with 2% as with 0.3% of glucose.

The inhibition of phosphorylase by glucose cannot be explained simply by competition between glucose

Table 7. *Inhibition by glucose of glycogen formation in muscle eluate; influence of adenylic acid in counteracting this inhibitory action of glucose*

Incubation period (min.)	Material added		Formation of glycogen from added glucose-1-phosphate	Inhibition of glycogen formation from glucose-1-phosphate
	Glucose (initial conc. %)	Adenylic acid (mg. added to 1 ml. eluate)	(% of theoretical)	(% of max. possible)
10	0.0	0.0	44	0
	2.0	0.0	1	98
15	0.0	0.0	46	0
	2.0	0.0	7	85
	2.0	1.5	30	35
	2.0	(1.5*)	8	83
	0.0	0.0 plus		
	Na lactate (initial conc. = 2%)	0.0 plus	34	26
	Na pyruvate (initial conc. = 2%)		24	48
20	0.0	0.0	53	0
	2.0	0.0	3	95
20	0	0	25	0
	0.3	0	15	40
	2.0	0	2	92
	0	1.5	53	0
	0.3	1.5	35	34
	2.0	1.5	35	34

* Adenosine-3-phosphate.

and glucose-1-phosphate for the enzyme. Further proof for this is given by experiments with potato phosphorylase. This phosphorylase, like other phosphorylases from plants, is not activated by adenylic acid. Competition between glucose and glucose-1-phosphate should exist in this system as well as in animal phosphorylases, and phosphorylase action should be inhibited. If, however, glucose inhibition is connected with activation by adenylic acid, no inhibition by glucose should be observed with potato enzyme. The results in Table 8 show that the latter alternative applies.

Table 8. *Inhibition by glucose of glycogen formation in eluate of potato juice*

Incubation period (min.)	Glucose added (initial conc. %)	Formation of glycogen from added glucose-1-phosphate (% of theoretical)	Inhibition of glycogen formation from glucose-1-phosphate (% of max. possible)
15	0.0	43	0
	2.0	41	5
30	0.0	58	0
	2.0	62	0

(d) *Nature of polysaccharide synthesized by phosphorylase.* The polysaccharide synthesized by animal phosphorylase is generally glycogen, that synthe-

sized by plant phosphorylase starch. This has been deduced by Cori *et al.* [1939] and by Hanes [1940] from the colour given with iodine, and has since been confirmed by X-ray spectrum analysis [Baer & Cori, 1941]. An exception to this rule is constituted by muscle phosphorylase, which acts on glucose-1-phosphate with formation of starch. We have been able to show that, prior to the formation of starch by muscle phosphorylase, a polysaccharide is formed which gives the yellow-pine colour with iodine typical of glycogen. This reaction cannot be demonstrated with fresh phosphorylase preparations acting at room temperature because starch formation in this case is too quick. If, however, the enzyme preparation is left overnight in the ice-box before it is allowed to act on its substrate, or if incubation is carried out at 0°, the yellow-pine colour reaction can be demonstrated after 5–10 min. of incubation. The colour becomes increasingly masked by the blue iodine reaction as synthesis proceeds further.

DISCUSSION

Good agreement has been found between the intensity of glycogen metabolism and the phosphorylase and phosphoglucomutase activity of various tissues. This finding favours the assumption that glycogen metabolism in all animal tissues follows the path described for muscle and liver by Cori *et al.* [1939]. An unexpectedly high phosphorylase activity and intense glycogen metabolism is shown by subcutaneous tissue.

In rat muscle almost no phosphorylase activity could be demonstrated immediately after birth. Muscle phosphorylase activity becomes considerable at the age of 14 days, when the muscles also begin to function normally. In brain, on the other hand, phosphorylase activity is already practically maximal at birth. Nevertheless, glycogen phosphorolysis in brain brei at this stage is very slow. The apparent discrepancy was found to be attributable to the lack of active phosphoglucomutase. When the mutase activity develops to normal, 14–20 days after birth, glycogen phosphorolysis too was found to become normal. This development of glycogen phosphorylation activity parallels the rise in O₂ consumption [Fazekas, Alexander & Himwich, 1941], and increases as the phosphatatic and glycolytic activities decline [Cohn & Kaplan, 1941; Epelbaum & Skvirskaya, 1940] with advancing age.

In poisoning with P, CCl₄ or CHCl₃, the glycogen metabolism of the liver is disturbed. In severe cases no glycogen at all could be found in liver. This is correlated with a marked fall in phosphorolytic capacity, apparently due to a decrease in the activity of phosphoglucomutase. The phosphorylase activity of liver in such cases is almost normal.

SUMMARY

1. A modified method for the preparation of phosphorylase by adsorption on $\text{Al}(\text{OH})_3$ is described. By this method, phosphorylase can be demonstrated in all animal tissues.

2. The phosphorylase activity and the intensity of the glycogen metabolism of a tissue are shown to be positively correlated. The same is true also of the phosphorolytic breakdown of glycogen.

3. Subcutaneous tissue possesses a highly active phosphorylase, and metabolizes glycogen at a high rate.

4. Phosphorylase is not found in the muscles of rats less than 10 days of age. In 14-day-old rats the enzyme is already comparatively active.

5. Brain tissue from new-born rats shows 60 % of the phosphorylase activity of the adult brain. Phosphoglucomutase in new-born rat brain, however, was either inactive or absent. This enzyme is the limiting factor in glycogen phosphorolysis up to 14–20 days after birth, after which time its activity approaches that found in the adult brain.

6. In intoxication by phosphorus, carbon tetra-

chloride and chloroform, liver phosphoglucomutase is inactivated, but phosphorylase activity is almost normal.

7. In adrenalectomized as well as in thyroidectomized rats no decrease in glycogen phosphorolysis by muscle could be demonstrated.

8. Some properties of the phosphorylase of muscle have been studied: on irradiation with ultra-violet light, or on heating to 55° for 20 min., complete destruction of the enzyme occurred. Adenylic acid diminished the inhibition caused by glucose in 2 % concentration. With 0.3 % glucose no lessening of inhibition was found when adenylic acid was added. With potato phosphorylase no inhibition of glycogen synthesis by 2 % glucose was found.

9. If synthesis by muscle phosphorylase is slowed down by low temperature or by ageing of the enzyme preparation, an iodine colour reaction such as is given by glycogen can be observed after 5–10 min. of synthesis.

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The Chemical Behaviour of Dehydro-*l*-ascorbic Acid *in vitro* and *in vivo*

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It is reasonable to assume that the function of *l*-ascorbic acid in the metabolism of the animal organism is that of a link in a co-ordinated chain of reactions. It might therefore be expected that, since ascorbic acid and dehydro-*l*-ascorbic acid constitute under certain conditions a thermodynamically reversible oxidation-reduction system, dehydroascorbic acid would become a component of

these co-ordinated reactions, although under prevailing conditions it may be present in too small quantities to be detected by the available technique. With this hypothesis in view one of us started some years ago an investigation on the behaviour of dehydroascorbic acid in the guinea-pig. It was then found that whilst ascorbic acid could be almost completely recovered from the organs and tissues