3. Streptomycin is not converted into streptomycin as efficiently as is glucose.

4. *N*-Methyl-L-[¹⁴C]glucosamine, added after the organism had grown for 60 hr., was incorporated into streptomycin in over 15% yield. The radioactivity was located largely in the corresponding part of the antibiotic molecule, but the L sugar was also converted into the streptidine moiety more efficiently than D-glucose.

5. It is suggested that the inversion of D-glucose to give L sugars proceeds by way of a hexitol-type intermediate.

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Creatine and Carbohydrate Metabolism

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In a study of the distribution of high energy phosphates in X-ray irradiated guinea pigs it was observed that a 24 hr. fast produced a marked rise in the liver creatine of control and irradiated animals. Although a considerable literature exists on the consequences of starvation on creatine distribution (Benedict, 1907; Hunter, 1928; Beard, 1943), so far as we are aware creatinuria is reported to occur in prolonged rather than short-term deprivation. It is generally accepted that the creatine arises as muscle protein is broken down (Mendel & Rose, 1911; Myers & Fine, 1913), but since in our experiments a significant elevation was obtained at a time when peripheral tissue breakdown is unlikely (cf. Addis, Poo & Lew, 1936*a*, *b*) we thought it worth while to investigate the mechanism of the effect.

METHODS

Animals. Guinea pigs were fed a liberal diet of cabbage and bran. Rats from the department strain of Wistar rats were fed a stock diet of rat cubes (Messrs Heygate and Son, Northampton). During starvation water was always available *ad lib*. Adrenalectomized rats were maintained on 1% saline. The completeness of removal of adrenals was checked at death. Thyroid deficiency was produced by giving 0.1% thiouracil in the drinking water for 6 weeks before testing. The glands were sectioned and examined *post mortem* by Dr R. V. Coxon and histological changes

associated with chronic thiouracil-induced hypothyroidism were found to be present.

Hind-limb perfusion. The rats were anaesthetized with ether. The viscera and testes were removed and the descending aorta was cannulated above the bifurcation. The animal was transected and the hind limbs were connected to the perfusion apparatus. The carcass was supported on a muslin ring in a glass funnel; this served to filter and collect the venous return. The funnel was covered with a Perspex lid. The perfusion fluid was warmed to 38° and the limbs were kept at about 38° by an infrared lamp. Perfusion fluid (60–100 ml.) was oxygenated with a Hooker oxygenator and recirculated by means of a pump designed by Dr E. H. Schuster for use in the Department of Pharmacology, Oxford. The perfusion fluid was blood diluted with an equal volume of Ringer-phosphate and contained 10 mg. heparin/100 ml. The blood was obtained from donor rats by cardiac puncture under ether. The perfusion fluid could be sampled and the pressure and flow rate measured as required.

Liver extract. The animals were killed by cervical fracture, the abdomen was opened and the liver dropped immediately into ice-cold 10% (w/v) trichloroacetic acid (TCA) and dispersed in a high-speed homogenizer. The residue after centrifuging was extracted twice with 6% (w/v) TCA and the combined extracts were made up to volume.

Blood extract. For creatine estimations the animals were decapitated, the blood was collected in 10% TCA and the precipitated protein re-extracted with TCA as above. For assay of glucose, blood was deproteinized in the cadmium sulphate reagent of Fugita & Iwataka (1931).

Muscle extract. The animals were anaesthetized with sodium pentobarbitone and the quadriceps femoris of both legs displayed. Samples (200–500 mg.) were quickly dropped into liquid air, then dispersed in 10% TCA and re-extracted as usual. Some specimens were powdered in a steel mortar before homogenization.

Urine samples. Pairs of rats were placed in metabolism cages and the urine was collected. Because of the formation of creatine from creatinine at room and body temperatures, experimental collections were always compared with those from the same animals under similar control conditions.

Creatine. This was estimated by the method of Ennor & Stocken (1953). Bound creatine (phosphocreatine) was given by the difference between free creatine and that obtained after hydrolysis of the neutralized TCA extract by heating for 10 min. at 65° with 0.1 N-H₂SO₄. Recoveries of creatine added to the samples were checked both before and after hydrolysis (cf. Barker, Ennor & Harcourt, 1950).

Liver glycogen. This was estimated by precipitation from the ice-cold TCA extracts by an equal volume of ice-cold ethanol. After coagulation, the precipitate was centrifuged, dissolved in N-H₂SO₄, hydrolysed for 2–2.5 hr. at 100° and the sugar estimated on a neutralized sample by the method of Hagedorn & Jensen (1923). The factor 0.927 was used to convert glucose into anhydrous glycogen.

Blood glucose. This was estimated by the method of Hagedorn & Jensen (1923).

Liver arginase. This was estimated by the method of Folley & Greenbaum (1946). No activators were used. The urea was converted into NH₃ by urease–Dunning tablets and the NH₃ estimated in the Markham (1942) apparatus.

Chromatography. Ascending chromatograms of the ether-extracted and concentrated liver extracts were run as described for amino acids by Consden, Gordon & Martin (1944) and for creatine and glycoeyamine by Ames & Risley (1948).

RESULTS

The effect of starvation on the creatine and phosphocreatine content of guinea pig livers is shown in Table 1. The total creatine level in fed guinea pigs appeared to be less than 12 mg./100 g. liver (wet wt.) in both male and female animals. A 12 hr. fast produced a rise in total creatine content which was maintained after 24 and 48 hr. starvation. The increase was also observed in X-ray-irradiated animals, and in all the groups there was a proportionate increase in bound creatine so that the per-

centage phosphorylated remained fairly constant. This constancy in the percentage phosphorylated, and the development of maximal colour within 20 min. in the Barritt reaction suggested that the increase in creatine-reacting substance was creatine itself, and this was confirmed using the specific creatine micro-organism (Ennor & Stocken, 1953), when 96–97 % of the total creatine-reacting substance was destroyed.

The proportionate increase in liver phosphocreatine when the total creatine content increased, and the stability of the adenosine triphosphate (ATP) level at this time (Rowles, 1951), suggested that the creatine–phosphocreatine system responded directly to the creatine concentration and was not limited by ATP.

The nitrogen content of guinea pig livers increased from 3 to 3.5 % (wet wt.) as a result of a 24 hr. fast, but the rise in creatine cannot be accounted for solely as a concentration effect. It has been shown that of many mammalian livers the guinea pig has the greatest ability for synthesis of creatine (Cohen, 1951), and one possible explanation for the rise could be an increased synthesis of creatine in the liver from nitrogen precursors made available in the course of gluconeogenesis. Chromatographic examination of the liver extracts, however, showed no qualitative difference in amino acid contents of the livers from fed and starved guinea pigs, and no obvious increase in possible amino acid precursors of creatine (glycine, arginine, glutamic acid). Development of the papers by the Jaffé reaction to demonstrate creatine-like compounds (Ames & Risley, 1948) confirmed the increase of creatine in starvation but failed to show any increase in glycoeyamine.

Since protein deprivation is known to affect liver enzymes (Miller, 1948, 1950), the concentration of liver arginase was compared in fed and starved guinea pigs (Table 2). The enzyme activity expressed on a nitrogen basis (236 and 244 units/g. N) was unaltered so that diversion of arginine metabolism towards creatine synthesis appeared improbable.

Table 1. *Mean creatine levels in the livers of fed and starved adult guinea pigs*

No. of animals and sex	Period of starvation (hr.)	Creatine (mg./100 g. wet wt. of liver)			Bound creatine as % of total
		Free	Total	Bound	
2 M	0	8.1	11	2.8	25
2 F	0	7.4	9.5	2.1	22
1 M	12	28	33	5	15
5 M	24	32	44	12	28
2 F	24	—	26	—	—
2 M	48	20	32	12	38
4 M*	24	34	48	14	29
4 F*	24	54	71	17	24
					Mean 26

* Starvation begun 6 days after X-ray irradiation with 1000 r.

Because these experiments provided no conclusive evidence regarding liver creatine synthesis in starvation, it was decided to investigate more closely the conditions under which creatine appeared in the liver. The work of Cathcart (1909) and many others had established that the creatinuria of fasting was prevented specifically by carbohydrate feeding. It was therefore decided to investigate simultaneously the creatine and glucose or glycogen levels in blood and liver and the urinary creatine excretion in animals in acute starvation in an attempt to discover the cause of the altered creatine distribution. For this purpose rats were used and it will be seen (Table 3) that the liver creatine level in rats is lower than that in guinea pigs, but a 24 hr. fast still produced an increase which was in fact detectable at 12 hr. and remained steadily elevated over the period of starvation studied. Simultaneous determination of blood creatine showed

that this also increased sharply as a result of 24 hr. starvation and then fell to a steady level about 30% above that in fed animals. The excretion of creatine appeared to reflect the blood creatine level, rising very steeply to a peak after about 30 hr. fasting and then falling somewhat. The liver glycogen levels were similar to those reported previously for male rats in starvation (Harrison, 1953) and the absence of TCA-extractable glycogen as 12–24 hr. (Bloom, Lewis, Schumpert & Shen, 1951) coincided with the rise of creatine in blood and liver. The re-appearance of glycogen, after 2–3 days starvation, did not reduce the liver creatine level as might have been expected if the glycogen level was the determining factor.

In a further series of experiments blood glucose and creatine levels in four female rats were simultaneously determined over a 30 hr. period of starvation (Table 4). Blood samples were obtained by cardiac puncture under ether. The blood glucose fell markedly between 6 and 24 hr. starvation, rising again by 30 hr. To avoid the complication due to the effect of ether on the blood glucose level the experiment was also carried out with a group of male rats, half the animals being killed to give the fed glucose and creatine levels and the remainder being deprived of food for 24 hr. before death. The creatine levels were elevated after 24 hr., and again there was a pronounced drop in blood glucose concentration. The results of these experiments and of those shown in Table 3 suggested that the fall in blood glucose level apparent after 24 hr. starvation might be the initiating factor in the appearance of increased liver creatine and creatinuria.

Table 2. *Arginase levels in the livers of fed and starved adult male guinea pigs*

Amounts recorded are for 100 g. wet wt. of liver. An arginase unit = the amount of enzyme liberating 1 mg. urea in 30 min. under the conditions described by Folley & Greenbaum (1946).

	Total creatine (mg.)	Arginase (units)
Fed	15.4	778
	10.0	660
	12.8	683
Starved	33.3	953
	43.1	830
	41.4	783

Table 3. *Tissue creatine levels in starved rats*

Three groups, A, B and C, of rats were used. The animals in group A had been adrenalectomized; those in groups B and C were controls. In each group urine was collected from a pair of rats from the same litter as those used for tissue analyses.

Period of starvation (hr.)	Weight loss (%) C	Liver glycogen (mg./100 g. wet wt.)		Liver creatine (mg./100 g. wet wt.)			Blood creatinine (mg./100 ml.) C	Period of collection of urine (hr.)	Urinary excretion of creatine (μ g./rat/hr.)		
		B	C	B	C	A			B	C	A
0	0	5250	5025	3.4	3.0	4.1	2.5	0–6	9	13	30
		5580	3855	4.2	3.5	4.8	3.4				
6	—	1720	—	3.6	—	5.6	—	6–12	14	—	35
		2380	—	2.8	—	4.0	—				
12	—	3	—	5.0	—	5.8	—	12–24	22	—	132
		4	—	5.3	—	5.7	—				
24	7	0	47	7.2	5.4	8.0	6.4	24–30	153	361	445
	6	0	10	7.4	5.2	6.7	6.3	30–36	193	—	505
								36–48	158	—	268
48	15	456	53	5.2	6.3	8.2	6.3	48–54	—	233	191
	11	890	26	6.1	4.7	8.3	5.5	—	—	193	—
72	17	—	332	—	5.2	8.6	3.4	72–78	—	147	—
	17	—	161	—	5.3	—	4.2	—	—	—	—
96	24	—	492	—	6.8	—	4.2	—	—	—	—
	26	—	252	—	5.9	—	4.0	—	—	—	—
120	27	—	640	—	5.9	—	4.6	—	—	—	—

Table 4. *Effect of starvation on blood glucose and creatine levels in rats*

Blood samples (0.5 ml.) were obtained from the female rats by cardiac puncture under ether. The male rats were killed to provide blood samples.

Sex	Period of starvation (hr.)				Period of starvation (hr.)			
	0	6	24	30	0	6	24	30
	Creatine (mg./100 ml. blood)				Glucose (mg./100 ml. blood)			
F	3.5	3.7	4.2	3.5	143	110	72	127
F	2.6	3.9	5.4	7.2	107	124	65	97
F	2.9	3.6	3.0	3.8	130	124	70	127
F	2.8	3.3	*	—	125	124	—	—
M	3.9	—	5.8	—	108	—	77	—
M	4.1	—	7.0	—	94	—	65	—
M	3.7	—	5.6	—	88	—	61	—
M	3.8	—	6.1	—	98	—	64	—

* Died from haemorrhage following cardiac puncture.

Table 5. *Effect of 24 hr. starvation on the creatine content of the quadriceps femoris of the male rat*

	Creatine (mg./100 g. wet wt.)			Percentage phosphorylated
	Free	Total	Bound	
	Fed rats			
	270	522	252	48
	226	431	205	48
	240	541	301	56
	217	422	205	49
	315	489	174	36
	289	463	174	38
Mean	260±38	478±48	218	46
	Starved rats			
	320	563	243	43
	296	480	184	38
	210	391	181	46
	277	564	287	51
	276	495	219	44
	303	464	161	35
Mean	280+38	493±65	213	43

As no evidence had been found for enhanced creatine synthesis in the liver after 24 hr. starvation, mobilization of creatine from peripheral sites was an obvious alternative explanation. Skeletal muscle creatine and phosphocreatine were therefore measured after 24 hr. starvation when the rise in blood and liver creatines was well established. After 24 hr. there appeared to be a decrease in the percentage creatine phosphorylated (Table 5) but the levels of free and total creatine were not significantly different ($P=0.5$). It is clear, however, that since the concentration of total creatine in muscle is a hundred times that in blood, a trivial change in the proportion of free creatine could, if released into the circulation, bring about a very marked increase in the blood concentration.

Because direct analytical techniques were inadequate to demonstrate either the origin of the labile creatine or the controlling factors in its

mobilization, it was decided to turn to perfusion experiments. With the co-operation of Dr R. B. Fisher and Mr D. Lindsay the possibility of an increased creatine leak from heart muscle in starvation was investigated by means of their perfused rat heart preparation (Bleehen & Fisher, 1954). With hearts from normal well-fed rats there was no difference in total creatine liberated into the perfusion fluid after 30 min. when galactose replaced glucose in the medium. Hearts from 24 hr. starved rats gave the same loss of creatine when perfused either with or without glucose in the medium as had been found for the normal hearts.

Since no demonstrable difference was obtained with the heart muscle, hind limb perfusions were undertaken. We are indebted to Dr E. Bulbring for teaching us the operative technique. In these experiments the limbs were perfused with re-circulating fluid and the venous outflow was sampled for glucose and creatine content. The hind limbs were weighed before and after the experiments (initial weights 60–100 g.) and haematocrits were measured on samples of the perfusion fluid. The initial and final volumes of blood, the volume removed in sampling and the rate of loss of water by evaporation from the animal and supporting muslin were measured so that it was possible to establish complete balance sheets for the water and creatine changes in the experiments.

The first runs demonstrated that glucose was removed from the circulating fluid by the preparation and that as the glucose concentration fell creatine appeared in the perfusion fluid (Fig. 1). In a typical experiment 58 mg. glucose disappeared and 2.5 mg. creatine appeared in the circulating fluid after 2 hr. Of the glucose disappearing, a control experiment showed that approximately 12 mg. were taken up by the red cells in 2 hr. Since the leakage of creatine under these conditions might be due to the progressively deteriorating preparation, reversal of the phenomenon was attempted by

Fig. 1. The effect of falling glucose concentration on the creatine level in rat hind limb perfusate. Initial volume of perfusion fluid 94 ml. Initial weight of hind quarters 100 g., final weight 113 g. Constant haematocrit 23%. $\bigcirc$, Glucose; $\times$, creatine.

Fig. 2. The effect of introducing glucose on the creatine level in rat hind limb perfusate. Initial volume of perfusion fluid 100 ml. Initial weight of hind quarters 90 g., final weight 101 g. Initial haematocrit 21.5%, final 24%. $\bigcirc$, glucose; $\times$, creatine. At 15 min. 150 mg. glucose added to perfusion fluid reservoir.

Fig. 3. The effect of sodium iodoacetate on the creatine level in rat hind limb perfusate. Initial volume of perfusion fluid 100 ml. Initial weight of hind quarters 103 g., final weight 116 g. Initial haematocrit 23%, final 25%. $\bigcirc$, glucose; $\times$, creatine. At 15 min. 18.6 mg. sodium iodoacetate and 50 mg. glucose added in 10 ml. perfusion fluid.

Fig. 4. The effect of dinitrophenol (DNP) on the creatine level in rat hind limb perfusate.

$\bigcirc$ — $\bigcirc$, glucose; $\times$ — $\times$, creatine. Control. 90 ml. perfusion fluid. Initial weight of hind quarters 69 g., final weight 98 g. Initial haematocrit 30%, final 39%. At 15 and 30 min. 50 mg. glucose added in 1 ml. perfusion fluid.

$\bigcirc$ — $\cdots$ — $\bigcirc$, glucose; $\times$ — $\cdots$ — $\times$, creatine. Initial weight of hind quarters 60 g., final weight 76 g. Initial haematocrit 25%, final 32%. At 15 min. 18.4 mg. DNP and 50 mg. glucose added in 1 ml. fluid; at 30 and 45 min. 50 mg. glucose added.

increasing the glucose concentration in the circulating fluid after an initial period at a low level (Fig. 2). The limbs were perfused for 15 min. with 100 ml. 'blood' unsupplemented by glucose; as much as possible of the fluid was then collected and 150 mg. glucose dissolved in it before reintroducing the 'blood' into the circulation. There was an immediate drop in creatine concentration in the perfusion fluid which was maintained as glucose utilization continued. This reversal of the creatine leak from the preparation was almost invariably seen when glucose was added to the perfusion fluid, but was most marked when both the blood and the hind limbs were from fed animals.

To demonstrate the importance of glucose utilization in the maintenance of muscle creatine the effects of inhibition of glucose oxidation were investigated. The introduction of sodium iodoacetate did not prevent glucose disappearance but produced a striking increase in creatine level (Fig. 3, cf. Fig. 2). At the completion of the experiment the limbs were completely rigid.

In an experiment with dinitrophenol (DNP) in which the circulating glucose level was kept constant by the introduction of 50 mg. of glucose in 1 ml. 'blood' at 15 min. intervals, creatine once more escaped into the circulation (Fig. 4). This result is in contrast to a similar control experiment without DNP, in which there was a constant creatine concentration, and calculation showed that 0.6 mg. creatine disappeared from the perfusion fluid. With DNP the limbs were slightly rigid at the end of the experiment.

As a result of the perfusion experiments it seemed that reversible loss of creatine from skeletal muscle could be produced by a fall in blood sugar from 200 mg./100 ml. to 50 mg./100 ml. In view of the influence of endocrines on carbohydrate metabolism, a few experiments have been carried out to investigate possible hormonal effects on creatine distribution. In these experiments creatine mobilization in starvation was followed by determining urinary creatine excretions in fed and fasted control and experimental animals.

Adrenalectomized rats were examined in some detail (Tables 3, 6). The creatine excretion from fed adrenalectomized rats tended to exceed that in the controls, as had been reported earlier by Bodansky & Duff (1936). After 24 hr. fasting the creatinuria was extremely high and this was paralleled by the rapidly and greatly elevated liver creatine level. In the one animal examined after 3 days' starvation the liver creatine level was still very high, although its creatine excretion had dropped sharply. Renal failure was tentatively assumed. These results with adrenalectomized rats were not surprising in view of the known disturbance in glucose metabolism.

Thyroparivic and hypophysectomized rats also

exhibited the creatinuria of starvation, the hypothyroid animals being rather more sensitive than the controls. The sensitivity of the hypothyroid rats was interesting, as Bodansky & Duff (1936) reported a creatinuria and decreased myocardial and skeletal muscle creatine levels in fed hyperthyroid rats.

Some experiments on both rats and guinea pigs were also carried out to investigate the effect of cold stress. Short exposure to the cold had no effect on either fed or starved animals (Table 7), whereas Browne, Karady & Selye (1939) found that 24 hr. exposures produced a creatinuria.

Table 6. *Effect of hormonal withdrawal on urinary creatine levels of rats*

Urine was collected for 6 hr. Results are expressed as $\mu\text{g. creatine/rat/hr.}$ The period of starvation was 24 hr.

Controls		Adrenalectomized	
Fed	Starved	Fed	Starved
4	179	20	469
3	234	20	518
8	265	12	597
2	202		
2	253		
Hypophysectomized		Hypothyroid	
Fed	Starved	Fed	Starved
5	162	53	499
4	296	8	360
36	180		
26	83		

Table 7. *Effect of cold stress on liver creatine*

Stress was applied by keeping rats for 1 hr. and guinea pigs for 1.5 hr. in the cold room at 2°, the light being kept on. Animals were killed directly after stress. Control animals were kept in another room. Food was removed from both groups for the stress period.

Condition	Wt. of animal (g.)	Liver creatine (mg./100 g. wet wt.)	Liver glycogen (g./100 g. wet wt.)
Fed rats			
Control	160	3.4	4.0
	138	4.3	4.0
	119	5.2	2.6
Stressed	200	4.4	3.4
	128	5.3	2.6
	122	5.5	3.3
	120		2.9
Rats starved for 24 hr.			
Control	200	5.8	—
	200	8.0	—
Stressed	200	6.2	—
	200	8.4	—
Fed guinea pigs			
Control	530	7.2	1.1
Stressed	510	7.1	2.6

DISCUSSION

The experiments reported here followed the finding of a markedly increased liver creatine content in starved guinea pigs, an observation which has also been made by Ennor (personal communication). The creatine content of the livers of fed guinea pigs corresponded to that reported by Ennor & Rosenberg (1952) and is very low; 24 hr. fasting produced a threefold increase in total creatine content and a proportional increase in phosphocreatine, so that the amount of this form of high-energy phosphate showed an absolute increase. Only two simultaneous determinations of blood and liver creatines were performed with guinea pigs (the values in mg./100 g. or ml. were—fed, liver creatine 3 and 8; blood 2 and 2; starved, liver creatine 27 and 44; blood 2 and 5), but it is interesting that in this species there was a concentration of creatine in the liver in starvation, whereas in rats the liver creatine was never significantly different from the blood level and the increase on a nitrogen basis, after a 24 hr. fast, was less marked. In rats, therefore, the creatinuria which was established after about 24 hr. starvation was the most convenient index of creatine mobilization.

It is known (Pitts, 1934) that in dogs a small rise in plasma creatine produces a creatinuria and if the same consideration holds for rats this is adequate to explain the observed increased creatine excretion. Nevertheless, some derangement of kidney function as a result of starvation cannot be excluded as a contributory factor.

Although the increase in guinea pig liver creatine and the creatinuria in rats was striking, nevertheless the absolute quantities involved are very small. In a 200 g. rat the creatine excreted in the 30 hr. following withdrawal of food is not more than 0.4% of the total muscle creatine (muscle provides 45% of the body weight) and neither in rats nor in guinea pigs was any change in muscle creatine detected between fed and 24 hr. starved animals.

In explaining the cause of creatine mobilization many workers have concluded that carbohydrate metabolism is an important factor, but some of the experimental results are conflicting. Thus, Hobson (1939) found that a diet rich in carbohydrate was associated with a creatinuria, whereas Mendel & Rose (1911) prevented the creatinuria of starvation by feeding a carbohydrate diet. Factors contributing to the divergence of results could be the lack of sensitivity in the method employed for assay and failure to minimize the conversion of creatinine into creatine during the time between voiding and analysis of the urine.

The intimate relation between glucose and creatine is directly supported by the coincidence of

creatinuria with a fall in blood sugar below 100 mg./100 ml. and would explain the greater creatine excretion in starved adrenalectomized rats. Tissue analyses did not reveal the origin of the creatine, but while perfused heart failed to show any response to differences in availability of glucose, the hind limb preparation was quite sensitive. Rat skeletal muscle lost creatine reversibly with a fall in circulating glucose from 150 to 50 mg./100 ml., and factors known to interfere with glucose utilization, although possibly affecting creatine phosphokinase itself (Ennor & Rosenberg, 1954), produced a loss of creatine from muscle. With the perfusion fluid used in these experiments it was not possible to follow changes in blood potassium and phosphate levels, although these might be expected to parallel the creatine transport.

The reversible dependence of muscle creatine on the blood glucose level indicates that creatine can be released from this tissue in conditions of sudden carbohydrate shortage. It is suggested that the reduction in availability of oxidizable substrate will reduce the amount of creatine phosphorylated and the resultant increase in cellular creatine concentration will allow the liberation of small amounts of creatine into the circulation. In view of the reversible loss of creatine in hind limb perfusion experiments it is likely that liberation of creatine precedes that classically associated with muscle protein breakdown.

Disturbances in carbohydrate metabolism may also explain some of the effects on creatine distribution which have been reported in adrenalectomized animals. Our fed adrenalectomized rats, which were not used until at least 1 week after operation and which were maintained on saline, ordinarily had a slightly elevated liver creatine and creatinuria. No muscle creatine estimations were performed, but Conway & Hingerty (1953) have reported an increase in muscle phosphocreatine 5 days after adrenalectomy accompanied by a reduction in glucose 6-phosphate and an increase in ATP.

The influence of the parathyroids was not investigated, but Imrie & Jenkinson (1932) and Brown & Imrie (1932) have shown in cats that the phosphate retention following creatine administration is increased by parathormone administration, and that in thyroparathyroidectomized animals phosphocreatine resynthesis in muscle after stimulation is much reduced.

An additional factor influencing the release of creatine might be an alteration in electrolyte balance. Our experiments with hypertonic perfusion fluids were inconclusive since glucose utilization was impaired, but it is possible that some of the results reported by Beard (1943) can be explained on the basis of electrolyte imbalance and/or tissue dehydration.

SUMMARY

1. A two- to threefold increase in guinea pig liver creatine occurs within 24 hr. after the withdrawal of food.

2. In rats the increase in liver creatine is less marked, but a pronounced creatinuria occurs coincident with a fall in blood sugar to 60–70 mg./100 ml.

3. Perfusion experiments have demonstrated that rat skeletal muscle creatine is reversibly sensitive to blood sugar levels.

4. The relation between creatine distribution, carbohydrate metabolism and electrolyte balance is discussed.

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Uridine Compounds in Glucuronic Acid Metabolism

2. THE ISOLATION AND STRUCTURE OF 'URIDINE-DIPHOSPHATE-GLUCURONIC ACID'

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We have previously reported that liver contains a nucleotide composed of uridylic acid, labile phosphate and glucuronic acid in equimolecular proportions, and that the incubation of this substance with *o*-aminophenol or menthol in the presence of broken-cell liver suspensions results in the formation of the corresponding glucuronides (glucosiduronic acids) (Dutton & Storey, 1951, 1953). The enzyme system in the liver suspensions and some properties of the nucleotide have been described (Dutton & Storey, 1954).

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The present paper deals with the isolation of the pure nucleotide and a study of its degradation products under various conditions. The results confirm an earlier suggestion (Dutton & Storey, 1953) that its structure resembles the uridine diphosphate compounds isolated by Caputto, Leloir, Cardini & Paladini (1950), Cabib, Leloir & Cardini (1953) and Park (1952*a, b*), and that it may be regarded as 'uridine-diphosphate-glucuronic acid'.

Since the new nucleotide appears to be a substrate in the enzymic process, throughout both this and the preceding paper (Dutton & Storey, 1954) we have used the term 'factor' rather than 'cofactor' or 'coenzyme', which imply a function which is, as yet, unproved.