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THE INFLUENCE OF ADRENAL CORTICOIDS ON PHOSPHATE AND GLUCOSE EXCHANGE IN MUSCLE AND LIVER IN MAN

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It has been shown previously that intravenous cortisol, or oral cortisone, causes a disappearance of inorganic phosphate from plasma, but that deoxycorticosterone has no such effect (Mills & Thomas, 1957). The disappearance thus appears to be effected by glucocorticoids rather than by mineralocorticoids. An attempt has now been made to locate the site of phosphate disappearance. Attention has been directed to two main metabolically active tissues, muscle, since venous blood from this tissue is relatively simple to obtain, and liver, since it is here that the major rapid changes in carbohydrate metabolism are said to be produced by adrenal glucocorticoids. The method mainly employed has been a comparison of the phosphate concentrations in blood or plasma entering or leaving these tissues. Measurements have also been made of the blood flow through muscle under the influence of cortisol. A preliminary account of these experiments has already appeared (Mills & Thomas, 1958*b*).

METHODS

Ten healthy male subjects aged 21–43 have been studied. The subject ate a light breakfast, but nothing thereafter, and at 11.30–12.00 hr (or earlier in two of the control experiments) sat down quietly while a nylon catheter was inserted into a deep vein of one forearm by the technique of Mottram (1955). The position was checked radiologically after insertion of a metal wire whose length equalled that of the catheter. Into a superficial vein of the opposite forearm was inserted a distally directed nylon catheter or Guest cannula. When the catheters were in position, the subject remained seated in an easy chair for the remainder of the experiment, occasionally standing to void urine. Control experiments differed from those with steroids only in that no injection was given.

Five minutes before beginning to collect a deep vein sample, an arterial occlusion cuff around the wrist was inflated to 200 mm Hg, and the sample was collected without venous stasis; Coles, Cooper, Mottram & Occleshaw (1958) have shown that blood thus collected is derived entirely from the deep tissues, consisting mainly of muscle. The blood was obviously considerably reduced as judged from the colour; on the four occasions when O_2 content was determined, it was 64, 72, 54 and 38% saturated. The opposite forearm was heated by immersion in water at 45° C or over for at least 5 min before collecting samples, which will be referred to as arterial blood; these were

always bright scarlet. Two pairs of blood samples were usually collected before intravenous injection of hormones and further pairs of samples at intervals of 10–30 min for up to 3 hr; the more frequent sampling was used over the period when blood phosphate was expected to fall steeply. In order to avoid the period of spontaneous change in blood phosphate concentration, samples collected before noon were ignored. In six experiments no injection was given. In two of these only the initial pair of samples was collected, and in four, serial samples of 'arterial' and muscle vein blood were collected for 68 min to 4½ hr.

When hepatic vein samples were collected the technique differed in that the subject lay supine, and arterial samples were collected from an indwelling needle in the brachial artery. A cardiac catheter was introduced in the usual way through an antecubital vein and manipulated under radiological control until its end lay in a hepatic vein, usually the right. Since it was considered unwise to leave this catheter *in situ* for more than 1½–2 hr, cortisol was injected before catheterization began and control venous samples were therefore not obtained, but two control 'arterial' samples were collected before hormone injection by puncture of a superficial vein of the heated forearm.

After removal of a sample for phosphate estimation on whole blood, blood samples were centrifuged immediately and the plasma was carefully pipetted off.

The hormones given, all intravenously and usually into one of the indwelling catheters, have been cortisol (hydrocortisone hemisuccinate, Upjohn, 133·7 mg, representing 100 mg free alcohol, or EF Cortelan Glaxo, in the same dose); deoxycorticosterone (glucoside, Ciba, 30 mg); aldosterone (racemic, monoacetate, Ciba, either one injection of 0·5 mg or two of 0·25 mg at an interval of 1½ hr); and prednisone (as hemisuccinate, U-6140, Upjohn, 33·4 mg.).

Blood flow through muscle was determined by the clearance of ^{24}Na, using the technique described by Kety (1949). Correction for recirculating isotope was made by control counts over a symmetrical position on the opposite limb. If it be accepted that ^{24}Na clearance is proportional to blood flow, then flow will be inversely proportional to half-clearance time.

Analytical methods

Inorganic phosphate, blood and plasma; Fiske & SubbaRow (1925).

Glucose, plasma; Fujita & Iwatake (1931), and also in some later experiments by the glucose oxidase method of Middleton & Griffiths (1957).

Oxygen and carbon dioxide in blood; Van Slyke & Neill (1924).

In earlier experiments phosphate was determined in plasma, in later ones in whole blood. When both measurements were made on the same sample, the blood concentration was about 4% less, which is probably largely referable to the smaller water content of whole blood. The concentration in both fell together under the influence of cortisol, indicating fairly rapid equilibration across the erythrocyte membrane.

RESULTS

The inorganic phosphate concentration in 'arterial' blood behaved in exactly the same way as that already described for plasma (Mills & Thomas, 1957). About an hour after injection of cortisol it fell steeply, reaching a minimal value in 2–3 hr. By contrast, when no injection was given, or when deoxycorticosterone was injected, the concentration remained steady or rose slightly. Aldosterone also was ineffective (compare Figs. 1 and 2 with Figs. 6 and 8 of Mills & Thomas, 1957).

The results of analyses of muscle-vein blood are shown in Figs. 1 and 2. Inorganic phosphate concentration in muscle-vein blood (or plasma) was below that in 'arterial' blood; it followed a parallel course in experiments in which arterial concentration did not alter much (controls, aldosterone, DOCG)

with sometimes a terminal narrowing of the A-V difference; when arterial concentration fell steeply (cortisol, prednisone), the muscle-vein concentration fell even more steeply, and the A-V difference widened.

Detailed results of all experiments are given in Table 1. Initially, phosphate concentration in arterial exceeded that in muscle-vein blood, by 0·062 mM ± 0·009 (S.E.M., 17 observations). In four control experiments, and after injection of aldosterone or deoxycorticosterone, this A-V difference changed little.

TABLE 1. Mean A-V phosphate differences across muscle and liver. Initial values are those before hormone injection. 'After hormone' are values when arterial phosphate concentration was falling rapidly (after cortisol or prednisone), or during the corresponding period after other hormones or in control experiments

				Mean A–V phosphate difference (mM)		
					After hormone	
Subject	Injection	No. of sets of observations	Period of time covered	Initial muscle	Muscle	Liver
A*	cortisol	8	141 (14)§	0·045	0·23	—
B	cortisol	10	164 (26)	0·04	0·13	—
B	cortisol	7	75 (−45)	—	0·135	+0·03
Cv	cortisol	4	55 (−28)	—	—	+0·015
G*	cortisol	5	119 (9)	0·15	0·125	—
H	cortisol	7	91 (−50)	—	0·10	−0·02
J	cortisol	5	51 (−92)	—	0·085†	+0·01
M*	cortisol	8	170 (49)	0·045	0·205	—
M*	cortisol	5	51 (−60)	—	0·115	−0·02
T*	cortisol	8	174 (54)	0·075	0·21	—
T	cortisol	11	242 (56)	0·055	0·115	—
A	prednisone	10	208 (24)	0·02	0·23	—
Cv	aldosterone	8	173 (22)	0·12	0·06	—
H	aldosterone	8	174 (21)	0·05	0·055	—
M	deoxycorticosterone	8	207 (23)	0·04	0·08	—
Cm	deoxycorticosterone	8	210 (27)	0·095	0·09	—
A	none	8	276	0·08	0·10	—
F	none	5	247	0·07	0·05	—
M	none	3	68	0·075	0·065‡	—
W	none	4	100	0·065	0·10	—
Bt	none	1	—	−0·015	—	—
T	none	1	—	0·035	—	—

* Analyses on plasma; all others on whole blood. † Observations only began when fall in arterial concentration was almost complete. ‡ A single pair of samples 68 min from the start. § Figures in parenthesis indicate time after start when hormone was injected.

Examples of each are shown in Fig. 1. The A-V difference likewise remained unaltered for about 1 hr after injection of cortisol or prednisone, which was usually carried out at about 13.00 hr. The arterial and venous concentrations then fell steeply and the A-V difference widened to 0·156 mM ± 0·015 (S.E.M., 10 expts.). This mean differs significantly ($P < 0·01$) from the mean initial A-V difference of 0·062 mM. A typical result is shown in Fig. 2 and all the mean A-V differences are included in Table 1. The mean A-V differences after cortisol and prednisone are calculated over the period when arterial concentration was falling linearly. In other experiments, when arterial concentration did not fall,

the calculation is made over a corresponding period, namely between 1 and 2 hr after injection of the hormone, or after the initial samples when no injection was given. In one exceptional experiment, on subject G, the A-V difference did not rise, but the initial A-V difference was very large, and was derived from only a single pair of samples; there was insufficient material to

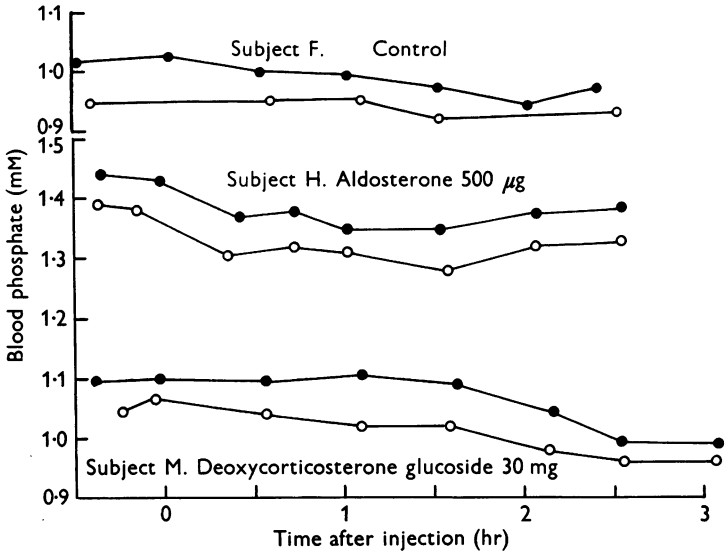

Fig. 1. Phosphate concentrations in 'arterialized' (●) and in muscle-vein (○) blood before and after intravenous injection of aldosterone and of deoxycorticosterone, and in a control experiment without injection.

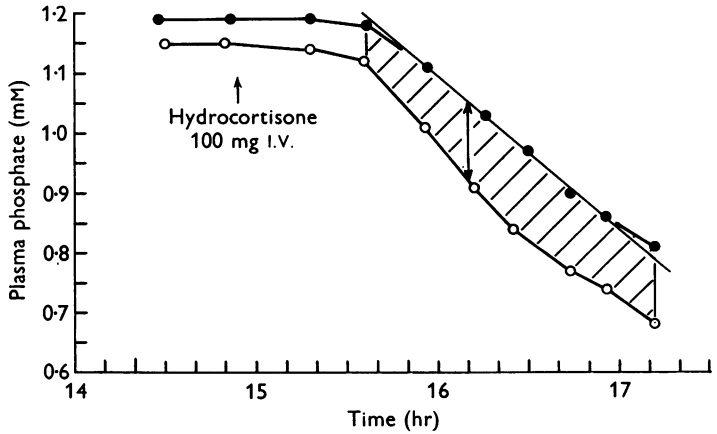

Fig. 2. Plasma phosphate concentration in 'arterialized' (●) and in muscle-vein (○) blood before and after intravenous injection of cortisol. The straight line fitted to the falling arterial concentration is used in calculating the rate of phosphate disappearance, and the shaded area in calculating the amount taken up by muscle. Subject B.

repeat the analyses, but this experiment has nevertheless been included in the means.

The difference in the effect of cortisol and prednisone on the one hand, and aldosterone, deoxycorticosterone and control experiments on the other, can be brought out in two further ways. Table 2 shows the lowest inorganic phosphate concentration observed in muscle-vein blood in the course of each experiment, and the differences are notable; in several cortisol experiments the lowest concentration was found in the last sample collected, so the minimal value may have been reached after the end of the experiment. A further statistical calculation has been made of the results of those experiments in which pairs

TABLE 2. Minimal values of inorganic phosphate concentration in muscle-vein blood

Subject	Injection	Minimal blood phosphate (mM)	Subject	Injection	Minimal blood phosphate (mM)
A	cortisol	0·42	A	prednisone	0·605
B	cortisol	0·68	Cv	aldosterone	0·945
B	cortisol	0·70	H	aldosterone	1·28
G	cortisol	0·79*	Cm	deoxycorticosterone	0·90
H	cortisol	0·75	M	deoxycorticosterone	0·96
J	cortisol	0·745	A	none	1·13
M	cortisol	0·55*	F	none	0·92
M	cortisol	0·62*	M	none	0·95
T	cortisol	0·69*	W	none	0·88
T	cortisol	0·655			

* Analyses on plasma.

of samples were collected before and after hormone injection, or in the second hour of control experiments. For seven cortisol and prednisone experiments the mean increase of A-V difference was 0·116 mM ± 0·031 (S.E.M.), whereas for eight experiments with no injection, or after injection of aldosterone or deoxycorticosterone, the mean increase was 0·001 mM ± 0·01 (S.E.M.). The difference between these means is highly significant ($P < 0·01$).

The widening of the A-V difference as the arterial phosphate concentration falls suggests that uptake by muscle is a major cause of the fall of plasma concentration. Blood contains, however, much more combined than inorganic phosphate, and changes in inorganic phosphate concentration could be due merely to internal redistribution between these fractions. Before muscle can be held responsible for the changes it is therefore necessary to show that alterations in plasma or whole blood phosphate concentrations do not represent redistribution due to removal of O_2 from blood, or to addition of CO_2.

Blood samples removed by venepuncture were therefore equilibrated with different gas mixtures, and the phosphate concentrations determined. The results are shown in Table 3, and it will be seen that changes in gas tension produce only minor changes in phosphate concentration in whole blood, but that removal of O_2 causes a rise of concentration in plasma, indicating

presumably movement of phosphate out of erythrocytes. For this reason all our later determinations were performed upon whole blood. Changes of CO_2 tension over the physiological range were without apparent effect.

Changes in A-V difference only indicate changes in muscle uptake if blood flow is unaltered. Muscle blood flow before and after cortisol injection has therefore been determined in a number of experiments, with the results shown in Table 4. In the first experiment, identical sites for ^{24}Na injection were used

TABLE 3. Changes in plasma and blood phosphate concentration produced by equilibrating blood with different gas mixtures

Sample	Gas content (vol/100 ml.) CO_2	Gas content (vol/100 ml.) O_2	Phosphate concentration (mM) Plasma	Phosphate concentration (mM) Blood
1	42	9·1	1·16	—
	40	16·6	1·11	—
	44	19·6	1·06	—
2	32	3·5	1·35	1·18
	43	20	1·26	1·22
	46	4·3	1·32	1·20
	52	19·6	1·23	1·23

TABLE 4. ^{24}Na clearance from muscle; for explanation see text

Expt.	Subject	Muscle group	Half-clearance time (min) Control	Half-clearance time (min) After cortisol	Ratio Flow after cortisol / Control flow
1	T	Upper arm	16·5	11	1·5
		Forearm	15	10·7	1·4
		Thigh	23	25·8	0·9
		Shin	17	14·5	1·2
2	A	Forearm	9·8	11·4	0·9
		Thigh	12·8	17·9	0·7
		Shin	16·2	22·0	0·7
3	A	Forearm	8·8	9·4	0·9
	G	Forearm	14·5	16	0·9
	M	Forearm	10·8	13·5	0·8
	T	Forearm	13	11·2	1·2

before and after cortisol, but a month elapsed between the two sets of observations. In the second experiment cortisol was injected immediately after the first series of counts was completed, and the second series of clearance measurements was performed upon the symmetrical site on the opposite side of the body; background count over the original sites was reduced by the time of the second injections to the general background elsewhere over muscle. In the third experiment identical sites were used for isotope injections before and after injecting cortisol, namely the forearm muscles apparently drained by a muscle-vein catheter, but a larger amount of isotope was injected the second time, giving initial counts of 12000–18000 instead of 5000–7000 per half minute. This proved to be unnecessary, as background count over the injection site

was, by the time of the second injection, reduced to the level over the opposite forearm.

The initial counting covered a period of 35–40 min, after which the count was too near background for accuracy, and the count after the second injection of isotope covered the period of 80–120 min after the injection of cortisol. This is the period when blood phosphate concentration is usually falling rapidly, with a large A-V difference. In Experiments 1 and 2 no measurements other than those of ^{24}Na clearance were performed; but in Experiment 3 arterial and muscle-vein blood was collected from subject T, and urine from subjects A and G. Analyses indicate that the period of blood flow estimation coincided with the period of rapid fall of blood phosphate or of phosphate excretion.

Cortisol produced no consistent change in the muscle blood flow, which was subject to random variation similar to that recorded by other workers using the same method (Bishop, Donald, Taylor & Wormald, 1957), and smaller than that often observed by Mottram (1958) with a different method.

In order to assess the contribution to phosphate exchange made by the liver and viscera draining into the portal vein (splanchnic viscera), blood samples were collected from the hepatic vein. Control samples were collected from two subjects, and after cortisol injection serial samples were collected from the hepatic vein and brachial artery of five subjects, together with muscle-vein samples from four of them.

Owing to the delay of about 1 hr which elapses after injection before blood phosphate starts to fall, it was necessary to inject cortisol before starting catheterization, and the time occupied in introducing two catheters and an indwelling arterial needle was somewhat unpredictable. On one occasion (subject J) these operations were so slow that only the last 30 min of the fall in blood phosphate was observed; on another occasion (subject C) all tubes were quickly in position, but owing to a persistent nodal rhythm it was considered wise to withdraw them and only the first 30 min of the fall was observed; on the other three occasions samples were collected over a period of 50–90 min while blood phosphate was falling. The period covered in each experiment is indicated in Table 1, with the mean A-V differences from all experiments. The full results of one experiment are shown in Fig. 3. The A-V difference across muscle for subject J is less than usual, but blood phosphate concentration was already beginning to level out after its fall before sampling began.

A-V differences across liver and splanchnic viscera were always very small, and in fact negative in two out of five experiments. These differences of sign between different subjects are probably significant, since the sign was the same for every A-V difference in one experiment: that is to say, the liver and splanchnic viscera in each subject either consistently absorbed, or consistently released, phosphate. Even if allowance is made for a liver blood flow nearly

double that through muscle, the effect of the liver and splanchnic viscera upon blood phosphate concentration must be small compared with the effect of muscle. We have only two values for phosphate A-V differences across liver and splanchnic viscera without hormone injection, +0·025 and −0·012 mM.

Urine was collected from subjects injected with cortisol in three experiments of the present and fifteen of the previous (Mills & Thomas, 1957) series. As plasma phosphate concentration fell, the urinary excretion of phosphate fell too (Mills & Thomas, 1958*a*), so that the change in urinary excretion appeared to result from, rather than to cause, the fall of plasma concentration. After injections of aldosterone or deoxycorticosterone, which do not depress plasma phosphate, phosphate excretion does not fall (Mills & Thomas, unpublished observations).

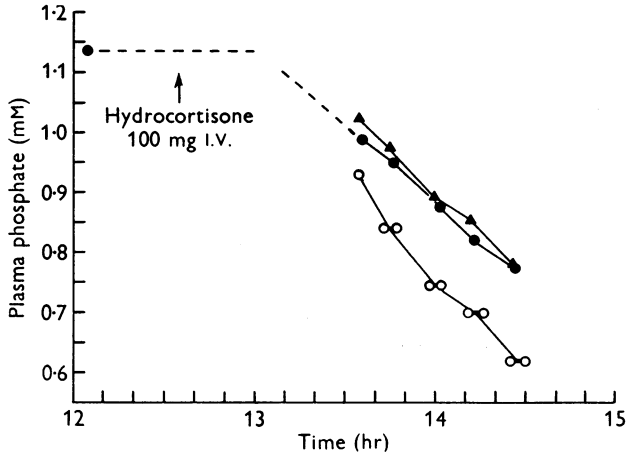

Fig. 3. Plasma phosphate concentration in arterial (●), muscle-vein (○), and hepatic-vein (▲) blood after intravenous injection of cortisol. The discontinuous line is the conjectured course of the arterial concentration over the period when no samples were collected. Subject M.

Since the disappearance of phosphate from blood as it traverses muscle is promoted only by glucocorticoids, it seems possible that it is associated with the various phosphorylations involved in carbohydrate metabolism, as for instance in the first stage of glucose uptake. Plasma glucose has therefore been estimated, both as total ferricyanide-reducing substances and, in later experiments, by the specific glucose oxidase method. The mean results are presented in Table 5. The figure for the initial A-V difference across muscle is the mean of all experiments, whatever the subsequent procedure. The mean figure after injection of cortisol includes experiments with hepatic vein catheterization, in which no samples were collected before injection of hormone. Since the initial A-V difference was widely variable, between 0 and 26 mg/100 ml., a further calculation (column 3) has been made of the mean change in A-V difference in the smaller number of experiments in which values before and after hormone

injection are available. It will be seen that the A-V difference across muscle tended to decline in the course of an experiment, whether or no cortisol or any other hormone was given. The hormones given do not appear to have influenced uptake of glucose by muscle. By contrast, arterial glucose concentration rose in all experiments after cortisol or prednisone injection, as was observed in many of the experiments of Mills & Thomas (1957); in control experiments and those in which aldosterone or deoxycorticosterone was injected, arterial concentration remained steady or tended to fall. Examples are shown in Fig. 4.

TABLE 5. Mean A-V differences in plasma glucose concentration (mg/100 ml.). For each subject a single mean difference was calculated from 1–4 pairs of blood samples before hormone injection, and a second mean difference from 3–6 pairs of blood samples while blood phosphate was falling rapidly after injection of cortisol or prednisone, or at the corresponding time in experiments with aldosterone or deoxycorticosterone and in controls. Values tabulated are the mean of results on all subjects, with number of experiments in parentheses

	A-V, muscle		Change of A-V, muscle		V-A, liver
Initial	10·0 (15)		—		—
After cortisol or prednisone	5·2 (9)		−3·4 (5)		9·6 (4)
After aldosterone	5·9 (2)	5·2 (7)	−3·6 (2)	−4·9 (7)	—
After deoxycorticosterone	3·2 (2)		−4·0 (2)		—
Controls	6·0 (3)		−6·3 (3)		—

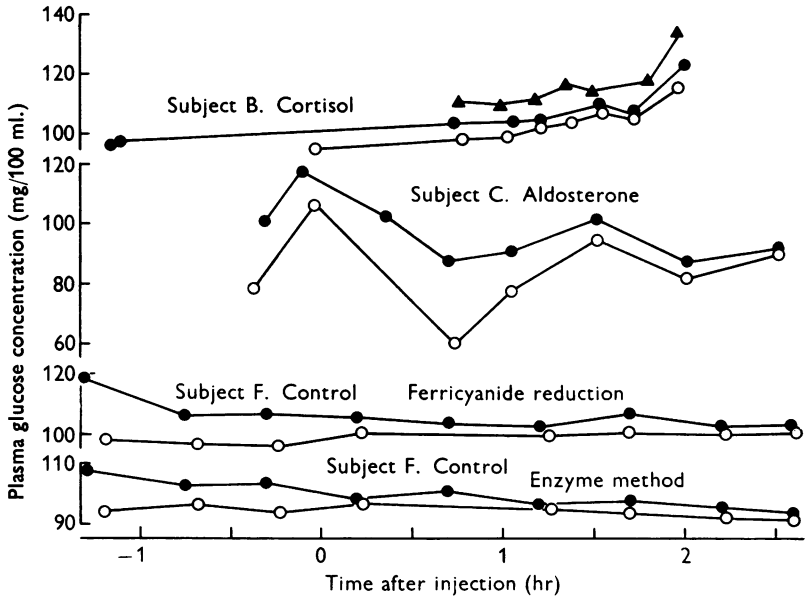

Fig. 4. Plasma glucose concentration in arterial (or 'arterialized') (●) and in muscle-vein (○) blood after intravenous injection of cortisol and of aldosterone, and in a control experiment without injection. Figures for hepatic-vein blood (▲) are included in the cortisol experiment, and for analyses by the enzyme method in the control; other analyses were done by ferricyanide reduction.

The mean A-V difference estimated by the glucose oxidase method did not differ from that estimated by ferricyanide reduction.

Many estimations were made of plasma K concentration. These will not be reported here, since we have observed no changes which we are willing to attribute to cortisol, and it may be that such changes as were observed form part of the diurnal rhythm described by Andres, Cader, Goldman & Zierler (1957).

DISCUSSION

Three points of special interest emerge from these observations. First, the inorganic phosphate concentration is consistently lower in blood draining resting muscle than in arterial blood. Secondly, when cortisol depresses arterial concentration, A-V difference across muscle widens notably. Thirdly, the liver and splanchnic viscera contribute little to the changes of phosphate balance induced by cortisol.

The loss of inorganic phosphate as blood traverses muscle, evidenced by a positive A-V difference, has persisted in control experiments for up to 4½ hr despite a steady urinary loss and a stable plasma concentration. It is not known whether this loss is balanced by addition of inorganic phosphate by some other organ, or whether it represents conversion of phosphate into an esterified form. An increase in ester phosphate would scarcely be detectable against the high total phosphate concentration in blood without using specific methods of estimation.

When cortisol depresses arterial inorganic phosphate concentration, the urine is not responsible for the loss, since excretion is less than before injection. The widening A-V difference across muscle, without consistent change in blood flow, makes it obvious that muscle is responsible for a substantial fraction of the loss. By making a few simple and reasonable assumptions, it is possible to calculate from the fall in blood phosphate concentration, and the A-V difference across muscle, the amount of phosphate disappearing from the extracellular fluid (e.c.f.), and taken up by muscle.

If phosphate equilibration is fairly rapid throughout the extracellular space, as Kleiber, Smith, Ralston & Jasper (1949) found upon a much larger animal, the cow, the total inorganic phosphate content of blood and extracellular fluid is the product of the volume of this fluid, which will be referred to as b.e.c.f., and the phosphate concentration in plasma or blood. Loss of inorganic phosphate over any specified period is then found by multiplying this volume by the drop in blood concentration, giving values of 60–120 μmoles/min, or a total of 3–9 m-moles.

Uptake by muscle is taken as blood-flow × A-V difference, and total uptake as A-V difference × area between plots of arterial and muscle-vein concentration against time (Fig. 2). Thus calculated, muscle uptake accounts for a loss of 70–190 μmoles/min, or a total of 5–18 m-moles. If allowance is made for the

resting rate of loss in traversing muscle, then the extra loss ascribable to cortisol approximates to the total loss from blood and extracellular fluid.

The calculation of loss from b.e.c.f. involves a knowledge of the volume of this fluid, which has been taken as inulin space when known (subjects M and T, Longson, Mills, Thomas & Yates, 1956), or as 17% of body weight (Keith, 1953), with an addition of 2 l. for erythrocytes. The calculations of loss in traversing muscle are less reliable, since it must be assumed that metabolic activity of forearm muscles is quantitatively similar to that of other muscles. Previous figures for flow (2·9 ml./100 g forearm muscle/min, Mottram, 1955), and for total body muscle mass (von Bardeleben, 1912), have been used. Very little skeletal muscle was active, presumably only the respiratory muscles, but the phosphate exchange here may well differ from that in resting muscle. The calculation would be invalidated if muscle blood flow diminished considerably after cortisol injection. The ^{24}Na clearances (Table 4) show, however, that any changes are modest in size and random in direction. Random changes of this magnitude have been observed by other workers using the ^{24}Na clearance, and larger random changes have been found by the plethysmographic method (Mottram, 1958).

It has been assumed that the deep forearm vein from which blood was collected drains only muscle. Coles *et al.* (1958) have shown that samples collected in this way are uncontaminated with blood from superficial veins. Mottram (1955) calculates that 10% of the blood collected may come from tissues other than muscle, of which only bone would be expected to contribute appreciably to phosphate exchange. Since after cortisol injection much more than 10% of the inorganic phosphate content of arterial blood usually disappeared in transit to the deep forearm veins, bone could not be responsible even if blood emerged from bone totally devoid of inorganic phosphate.

Many minor potential sources of error will also be apparent, but in general they lead to an underestimate of the contribution made by muscle. Taking into account all the possibilities of error in estimating inorganic phosphate exchanges, it seems that the amount lost in traversing muscle equals, or even exceeds, the total loss from blood and extracellular fluid after injection of cortisol.

The contribution of liver and splanchnic viscera to phosphate exchange may be assessed quantitatively in the same way if one assumes a total liver blood flow of around 0·02 l./min/kg body weight (Bearn, Billing & Sherlock, 1951). This contribution was sometimes positive, sometimes negative, and always small. Its magnitude is uncertain, as it is derived from very small A-V differences.

The phosphate changes we observed were induced by glucocorticoids and not by mineralocorticoids. The most consistent and rapid metabolic effect of glucocorticoids is an increase in liver glycogen (Long, Katzin & Fry, 1940;

Thorn, Koepf, Lewis & Olsen, 1940; Reinecke & Kendall, 1943). Our discovery that after injection of these steroids phosphate disappears from blood during its passage through muscle, and not through liver, was therefore most unexpected. No consistent changes in glucose uptake have been found to accompany the phosphate uptake, and at present the interconversions undergone by the phosphate, and its connexion with metabolic changes, are entirely unknown. It is not even known whether it is retained in muscle, or whether it is esterified or otherwise combined and returned to the blood in this form. The positive A-V difference which we have always found under control conditions suggests that the phosphate is returned to the blood in some combined form, but there are other possibilities: the total time span covered by our experiments is only from 12 noon to 5 p.m., and there may well be a movement in one direction at one time of day and a reverse movement at some other time, as has been shown for K by Andres *et al.* (1957). An abrupt disappearance of phosphate from the blood early in the morning is a familiar aspect of the diurnal renal rhythm (Stanbury & Thomson, 1951; Mills & Stanbury, 1952), but it is not known in what form or in what tissues the phosphate disappears, nor whether the changes are related to adrenocortical activity.

SUMMARY

1. Inorganic phosphate concentrations in plasma and in whole blood are approximately the same, and fall in a similar manner after intravenous injection of cortisol.

2. Under control conditions, in the early afternoon, inorganic phosphate concentration in muscle-vein blood or plasma is below that in arterial blood or plasma. This A-V difference widens when the arterial concentration is falling after injection of cortisol or prednisone. In experiments without injection, or after injection of aldosterone or deoxycorticosterone, arterial concentration and A-V difference across muscle remain fairly constant.

3. Calculation shows that uptake in muscle is roughly equal to the phosphate which disappears from blood under the influence of cortisol or prednisone.

4. Inorganic phosphate concentration in hepatic-vein blood is very similar to that in arterial blood when the concentration is falling after the injection of cortisol. The liver makes at most a minor contribution to phosphate exchange.

5. Glucose concentration in arterial plasma usually rises after the injection of cortisol or prednisone, and remains constant or falls after injection of aldosterone or deoxycorticosterone and in control experiments. The A-V difference across muscle narrows somewhat in all experiments.

6. The metabolic processes underlying phosphate uptake by muscle are discussed.

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