

The Influence of Adrenalectomy on Muscle Constituents

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The following studies were initially undertaken to investigate the quantitative relations of potassium changes in blood and muscle after adrenalectomy in the light of the work done on the nature of potassium accumulation (Boyle & Conway, 1941). In the course of the investigation the mean values of the known and quantitatively important substances in muscle and plasma were determined. These include Na, K, Ca, Mg, Cl, HCO_3 , pH, water, inorganic phosphate, total phosphate, urea, and total molecular concentration in plasma; Na, K, Ca, Mg, Cl, water, carnosine, anserine, total phosphate, total acid-soluble phosphate, adenosinetriphosphate, phosphocreatine glucose-6-phosphate, glucose-1-phosphate, fructose-6-phosphate, fructose-1:6-diphosphate, triose phosphate, phosphoglyceric acid, and phosphopyruvic acid in muscle. The results were used to calculate the change in non-diffusible material in the muscle fibre as well as the sum of the electrical charges thereon (η and ϵ values) following adrenalectomy with a view to interpreting more fully the cation changes. While this was the primary object of the present study, the greatly altered concentrations of the hexose esters appear to provide results of interest with respect to the changes in the intermediary metabolism of carbohydrate.

METHODS

Treatment of animals

Albino rats, almost all of the Wistar strain, were used as experimental animals; young animals weighing about 70–100 g. were chosen as these showed more marked deficiency conditions than older rats, which have some accessory adrenal tissue not removed at operation and elaborating sufficient hormone to relieve the symptoms (Verzár, 1939). These rats were maintained on a low Na diet following adrenalectomy, controls being maintained on the same diet for a corresponding number of days.

Removal of glands. The rat was anaesthetized by means of an ether-soaked pad of cotton-wool in a large glass jar and tied out back upwards on a flat board. The hair of the back was then clipped short and a cut about 2–3 cm. long made across the middle of the back. The skin was then retracted and a small incision about 5 mm. long made through the muscles a little below the ribs. The gland was then located on the upper pole of the kidney and removed by quickly tearing away with forceps; no appreciable bleeding occurred in this method and consequently ligatures were

unnecessary. After removal of both glands in this manner the severed tissues were stitched in position and the wound and adjacent skin region painted with diluted iodine solution. Following the operation the rats were maintained at a temperature of about 65° F. in a thermostatically controlled room. Four or five days after the operation they were taken for the blood and muscle analyses, but within that interval they occasionally died.

Sampling of blood and muscle. The estimations of the various muscle constituents were carried out on the leg muscles, excised immediately on killing the animal. Blood was collected by bleeding directly into a centrifuge tube (under light ether anaesthesia) except when required for pH, bicarbonate, and chloride determinations, when it was collected without exposure to air under liquid paraffin through a cannula in the abdominal aorta under ether anaesthesia; or when required for total molecular concentration determination, when a few drops were taken from the tail vein.

Chemical estimations. Inorganic. Sodium and potassium were estimated by colorimetric modifications (Boyle, Conway, Kane & O'Reilly, 1941) of the methods of Salit (1932) and Shohl & Bennett (1928) respectively, with preliminary ashing as described by Fenn & Cobb (1934). Magnesium was determined by a hydroxy-quinoline method (Cruss-Callaghan, 1935) and calcium was determined by the method of Kramer & Tisdall (1925) as modified by Clark & Collip (1925). Chloride and bicarbonate were estimated in plasma by micro-diffusion methods (Conway, 1939) and muscle chloride was estimated by a modification of the method described for amphibian muscle (Conway, 1939) involving the diffusion of the chloride from muscle slices into isotonic Na_2SO_4 , removal of protein by sodium tungstate and H_2SO_4 , and estimation of chloride in the filtrate by the micro-diffusion method. The *water content* was found by drying overnight at 105° in platinum crucibles prior to ashing for Na and K estimations. The pH of the blood was measured by means of the Beckman meter. In determining the total molecular concentration of the blood a new micro-diffusion technique was used requiring only 3–4 mg. for an individual determination, so that estimations could be carried out on the same rat before and after adrenalectomy by removing a few drops of blood from the tail vein. This method will be described in detail elsewhere. The results after adrenalectomy are given here as a percentage of the normal value.

Organic. Urea was estimated by the Conway (1939) method and carnosine and anserine were determined by the methods of Zapp & Wilson (1938). The various compounds occurring in the carbohydrate cycle were estimated after a fractionation procedure described in the following section, which is a combination and modification of the methods of Le Page & Umbreit (1943), Needham & van Heyningen (1935), Eggleton & Eggleton (1929) and Stone (1943).

Extraction of muscle and fractionation of extract

The rat is killed by a sharp blow on the head and about 2-3 g. muscle (excised within a few seconds of the animal's death) are dropped into 25 ml. ice-cold trichloroacetic acid in a mortar containing a little quartz sand and a pestle (all chilled in refrigerator, and weighed), ground immediately, reweighed, and then ground very finely. The solution is poured into a chilled 25 ml. centrifuge tube and quickly spun, using ice-water to jacket the tube, then poured through a small dry filter into a second chilled 25 ml. tube accurately graduated at 15 ml. until the graduation is reached, the remainder of the extract being filtered into another dry tube and left aside in the refrigerator. 1 ml. 25% Ba acetate is added to the solution, which is then neutralized by addition of conc. NaOH (phenolphthalein indicator), the tube and contents being kept cool by crushed ice. The end-point is attained if necessary with 10% trichloroacetic acid or dilute NaOH and the tube is allowed to stand for 1 hr. in the refrigerator, then centrifuged (the tube again being jacketed with ice-water) and washed once with about 2 ml. of an ice-cold Ba trichloroacetate solution (neutral) prepared by carrying out a blank procedure. The Ba precipitate, which gives fraction 1, is left aside in the refrigerator. (From the point of excision of the muscle until the addition of the Ba acetate all the procedures must be carried out as quickly as possible, all necessary reagents, chilled tubes, ice-water, etc., being previously prepared and ready to hand. With some practice it is possible to precipitate fraction 1 within 10 min. of the death of the animal.)

To the centrifugate and washings in a 150 ml. beaker, 5 vol. of ice-cold 95% (w/v) aqueous ethanol (neutral) are added. The end-point is adjusted to neutral if necessary and the mixture allowed to stand in the refrigerator for 3 hr. The precipitate which forms is collected by centrifuging successive portions in a 50 ml. pointed centrifuge tube at high speed (3000-4000 r.p.m.) washed with ice-cold 80% (w/v) aqueous ethanol (neutral) and left aside in the refrigerator. This precipitate gives fraction 2.

The centrifugate and washings from this procedure are concentrated to a small volume *in vacuo* at 35-40° (fraction 3).

The Ba and ethanol precipitates are each dissolved in about 2 ml. 2N-HCl and the Ba is removed by adding 1-2 drops conc. H₂SO₄ and centrifuging. The centrifugates and washings (BaSO₄ precipitates are washed with 1 ml. distilled water) are taken in specially graduated tubes, neutralized with strong NaOH solution, and made up to 5 ml. The temperature is kept low during addition of H₂SO₄ and neutralizing by immersing the tubes in crushed ice. Fraction 3 is concentrated to about 2-3 ml., the Ba removed by H₂SO₄, neutralized, and made up to 5 ml.

The following estimations on the various fractions are then carried out as required:

Original extract. Total phosphate, phosphocreatine, triose phosphate, phosphoglyceric acid.

Fraction 1. Total phosphate, adenosinetriphosphate, fructose-1:6-diphosphate, pentose.

Fraction 2. Total phosphate, total hexose esters, glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate, phosphopyruvic acid, pentose.

Fraction 3. Total phosphate (not further fractionated).

The recovery of total acid-soluble phosphate from the three fractions is about 95%.

Estimation of constituents in fractionated muscle extract

Inorganic phosphate was determined by the method of Fiske & Subarow (1925); phosphocreatine and total phosphate were measured by this method after hydrolysis and wet ashing procedures respectively. Adenosinetriphosphate was measured by the increase in free phosphate after 7 min. hydrolysis in N-HCl at 100° (Lohmann, 1929). Phosphoglyceric acid was estimated by the Rapoport method (1937) and triose phosphate as phosphoglyceric acid after oxidation by iodine (Meyerhof & Junowicz-Kocholaty, 1943). Fructose was estimated by Roe's method (1934) and pentose by the method of Mejbaum (1939). Phosphopyruvic acid was determined by measuring the pyruvic acid set free on hydrolysis for 60 min. at 100° in N-HCl (Lohmann & Meyerhof, 1934) by the method of Friedemann & Haugen (1943).

Hexose esters. The total hexose esters were determined by measuring the total phosphate remaining in fraction 2 after the residual unhydrolyzed phosphocreatine had been removed by HNO₃ and ammonium molybdate (Sacks, 1944) an allowance being made for the small amount of phosphopyruvic acid present. The increase in reducing value of the fraction after 7 min. hydrolysis in N-HCl at 100° affords a method of estimating the glucose-1-phosphate present, since glucose-6-phosphate and fructose-6-phosphate are already reducing without hydrolysis. Glucose-1-phosphate on hydrolysis had a reducing value 66.4% that of glucose (Le Page & Umbreit, 1943). The reducing value was determined by the Folin & Malmros (1929) method; the Shaffer & Hartman (1921) method as modified by Somogyi (1926) and the delicate method of Florin & Bosson (1929) were also tried but were less suitable for our purpose. Fructose-6-phosphate was estimated by determining the fructose present in this fraction. The difference between the total hexose esters and the glucose-1-phosphate + fructose-6-phosphate then gives a figure for glucose-6-phosphate.

In almost all cases the methods referred to were modified in details. In all the colorimetric methods extinction curves were prepared from suitable standard solutions of purified salts; the Pulfrich photometer or the Spekker photoelectric absorptiometer was used to measure the extinctions. The micro-cell of the Pulfrich photometer, which gives a 5 cm. layer with a volume of about 1.5 ml., was found particularly useful for low concentrations.

RESULTS

Muscle

The results for muscle are summarized as mean values in Table 1. From an inspection of Table 1 and considering the difference in the mean values and the standard deviations it would appear that the following substances are definitely increased after adrenalectomy (Table 2): potassium, magnesium, phosphocreatine, glucose-1-phosphate. Definite decreases are shown by sodium, glucose-6-phosphate, and fructose-6-phosphate, and probable decreases by total phosphate, chloride, and fructose-1:6-diphosphate.

The most striking percentage changes are shown by glucose-1-phosphate, glucose-6-phosphate, and

Table 1. Summary of data for normal and adrenalectomized rat muscle

(The figures in brackets give the number of experiments followed by the total number of rats in the group. Where no second figure is given in the brackets one rat only was used for each experiment.)

	Normal		Adrenalectomized	
	Mean (mmol./kg.)	s.e. of mean	Mean (mmol./kg.)	s.e. of mean
Potassium	101 (8, 15)	1.9	118.5 (15, 31)	1.3
Sodium	27.1 (10)	1.1	19.7 (19)	0.9
Calcium	1.56 (5, 7)	0.04	1.54 (6, 18)	0.06
Magnesium	11.0 (8, 14)	0.3	12.4 (8, 23)	0.4
Chloride	16.2 (5)	1.5	14.2 (9)	1.3
Total P	82.9 (7)	0.9	79.5 (11)	0.8
Acid-soluble P	61.1 (12)	0.8	60.7 (7)	1.6
Phospho-creatine-P	24.4 (11)	0.4	27.7 (12)	0.4
Adenosine-triphosphate-P	19.5 (8)	0.8	19.9 (9)	0.5
Total hexose monophosphate-P	10.04 (7)	0.87	5.67 (13)	0.46
Glucose-6-phosphate-P	6.42 (7)	0.85	2.06 (11)	0.16
Glucose-1-phosphate-P	2.74 (8)	0.21	3.68 (15)	0.19
Fructose-6-phosphate-P	0.94 (9)	0.06	0.34 (7)	0.03
Fructose-1:6-diphosphate-P	0.46 (11)	0.06	0.35 (7)	0.05
Triose phosphate + phosphoglyceric acid-P	3.55	—	3.55	—
Phosphopyruvic acid-P	0.073 (6)	0.004	0.077 (1)	—
Carnosine	1.36 (5, 14)	0.11	1.10 (7, 19)	0.12
Anserine	20.3 (5, 14)	4.9	23.5 (7, 19)	—
H ₂ O content (%)	76.8 (6)	0.3	77.4	0.3

In the above table millimolar values of adenosinetriphosphate and fructose-1:6-diphosphate are obtained by dividing the listed P values by 3 and 2 respectively.

Table 2. Substances in muscle increased or decreased after adrenalectomy

	% increase		mmol./kg. increase	
	Mean	Median	Mean	Median
Potassium	17.3	14.7	17.5	15.0
Magnesium	12.7	16.2	1.4	1.8
Phosphocreatine	13.5	13.5	3.3	3.3
Glucose-1-phosphate	34.3	19.8	0.94	0.57

fructose-6-phosphate, whilst the following substances show no appreciable change: adenosinetriphosphate, total acid-soluble phosphate, calcium. The distributions for substances definitely increased or decreased are shown in Fig. 1.

Plasma

The results are summarized in Table 3. Here also, on considering the difference of the means and

Table 3. Summary of data for normal and adrenalectomized rat plasma

(The figures in brackets have the same denotation as for Table 1)

	Normal		Adrenalectomized	
	Mean (mmol./l.)	s.e. of mean	Mean (mmol./l.)	s.e. of mean
pH (38°)	7.32 (4)	0.06	7.14 (4)	0.07
K	5.91 (9, 13)	0.18	9.16 (9, 13)	0.40
Na	138 (11)	1.8	128 (11)	3.0
Ca	3.11 (5, 8)	0.12	2.82 (5, 15)	0.10
Mg	1.46 (8, 14)	0.06	2.03 (8, 23)	0.09
Cl	110 (9)	0.9	104 (5)	1.0
HCO ₃ (as CO ₂)	22.4 (9)	0.5	20.1 (7)	0.3
Inorganic P	2.17 (7)	0.05	2.72 (7)	0.21
Total P	3.89 (7)	0.15	4.54 (7)	0.20
Urea	6.7 (7)	0.8	8.3 (7)	1.0
Total molar concentration taking normal value as 100	100 (7)	5.8	86 (7)	5.4
H ₂ O content (g./100 g.)	92.4	0.1	92.3	0.3

Table 4. Substances in plasma increased or decreased after adrenalectomy

	% increase		mmol./l. increase	
	Mean	Median	Mean	Median
Potassium	55.0	54.7	3.25	3.08
Magnesium	39.0	44.3	0.57	0.62
Urea	23.9	20.0	1.6	1.2
Total phosphate	16.7	19.3	0.65	0.73
Inorganic phosphate	25.4	29.4	0.55	0.62
	% decrease		mmol./l. decrease	
	Mean	Median	Mean	Median
Sodium	7.3	5.8	10.0	8.0
Chloride	5.5	4.6	6.0	5.0
Bicarbonate	10.3	11.1	2.3	2.5
Calcium	9.3	15.1	0.29	0.47

the standard deviations, we may list definite increases in potassium, magnesium, and-urea, and very probable increases of total phosphate and inorganic phosphate.

The following show a definite decrease: sodium, chloride, bicarbonate; and probable decrease of calcium (Table 4). The distributions for substances definitely increased or decreased are shown in Fig. 2.

Calculation of η and ϵ values

Here η = the 'idiomolar' value or sum in mmol. of the non-diffusible constituents in a number of muscle fibres which, for the normal animal, contained 1 l. of water.

For the computation of apparent values of η and ϵ we have the following equations (Boyle & Conway, 1941):

$$\eta/V = c - k_1 - \Sigma d_1, \tag{1}$$

$$\epsilon/V = k_1 - \Sigma d_1, \tag{2}$$

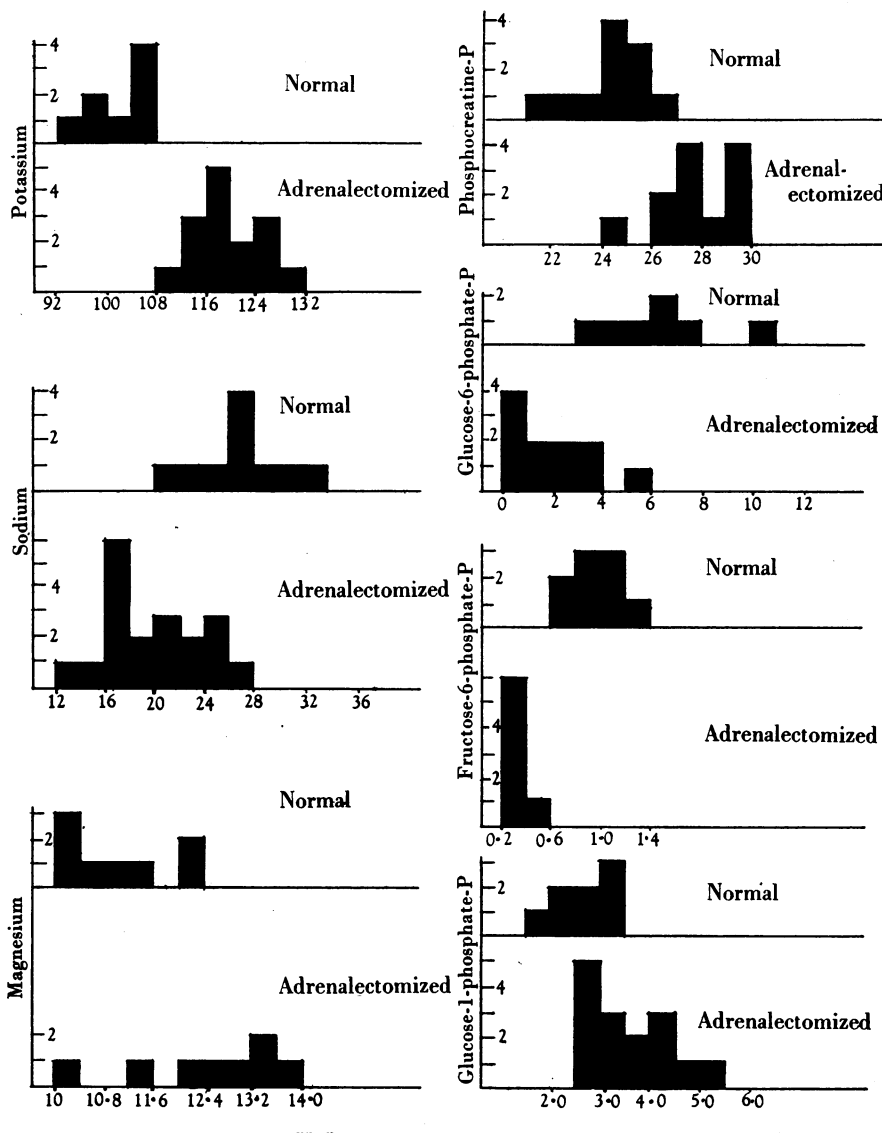


Fig. 1. Frequency distributions of muscle data for normal and adrenalectomized rats. The mean values are given in Table I. Abscissae (class intervals) are given in mmol./kg., and ordinates as numbers of observations.

ϵ = the electrostatic equivalent of η or the m-equiv. of base required to satisfy the surplus negative charges on η .

where V = volume of fibre water (taken as 1.0 for normal muscle, and in accordance with the above definition of η); c = total concentration of ions and

non-diffusible non-electrolytes in the plasma water; k_1 = mmol. K/l.; and Σd_1 = sum of the diffusible anions/l. fibre water.

To obtain values for k_1 and Σd_1 it is necessary to determine the extracellular space in muscle. This

respect to K, Cl and water exchanges in the muscle of live rats, but he found the product of K and Cl concentrations within the fibres to be only 0.5-0.6 times the product outside. The extracellular space in the tissue was determined by inulin. Now the

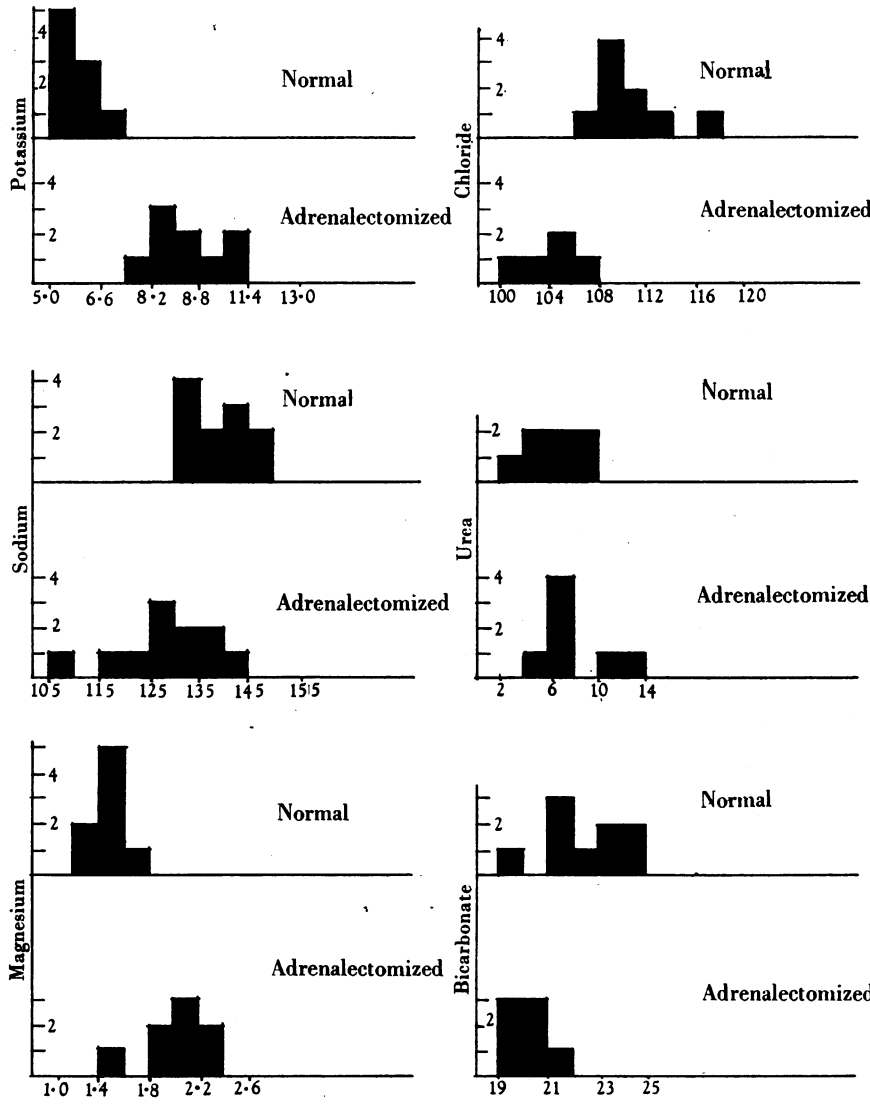


Fig. 2. Frequency distributions of plasma data for normal and adrenalectomized rats. The mean values are given in Table 3. Abscissae and ordinates as in Fig. 1.

may be done by assuming a Donnan relation for K and Cl across the fibres. Here the experiments of Wilde (1945) may be considered. He showed for nephrectomized rats that the general relationships predictable from the Conway theory (Conway & Boyle, 1939; Boyle & Conway, 1941) held with

inulin space does not give an exact measure of the Cl in the tissue outside the fibres, since inulin does not penetrate the red corpuscles in the capillaries or small blood vessels. For excised frogs' sartorii (Boyle *et al.* 1941) we have found that 2.3% of the whole tissue could be reckoned as

blood. Assuming a similar figure for mammalian excised muscle, the allowance necessary causes a very appreciable diminution in the apparent concentration of Cl within the fibre. When it is taken into account that the Cl in the interspace water is somewhat higher than in blood water (just as the Na and K to maintain a Donnan relation across the capillary walls may be expected to be lower) and may be represented from the available data as about 1.05 times, then the ratio of the products of K and Cl outside and inside the membrane becomes approximately 0.9. If the blood in the mammalian muscle were 4.6% instead of 2.3% of the whole we would obtain a ratio of 1.1. We may therefore suppose that the ratio is certainly higher than 0.6, and is at or near to 1.0, thus agreeing with the findings for frog muscle immersed in Ringer solutions with high KCl content (Boyle & Conway, 1941).

In calculating s or the interspace we may now consider the equation

$$k_1 l_1 = k_2 l_2, \quad (3)$$

where k_1 , l_1 and k_2 , l_2 are the K and Cl concentrations (mmol./l.) in the fibre and interspace water respectively.

The product $k_1 l_1$ may be expanded to

$$\frac{(k_0 - s k_2)}{(0.768 - 0.99s)} \times \frac{(l_0 - s l_2)}{(0.768 - 0.99s)},$$

where k_0 and l_0 are the mmol./kg. K and Cl muscle, 0.768 and 0.99 being the water content of 1 kg. tissue and of interspace fluid respectively.

The product $k_2 l_2$ may be taken as the same numerically as the similar product for plasma water, which from Table 1 (and taking the specific gravity of plasma as 1.03) may be written 6.2×116 . The value of s may then be calculated as 0.107. In a similar way for the adrenalectomized animal s is found to be 0.09.

From such data and similar calculations for adrenalectomized muscle the values of Table 5 are deduced, and from equations (1) and (2), the values of η and ϵ are 147 and 146 for the normal rat muscle and 120 and 175 for that of the adrenalectomized rat. In the calculation the V value for adrenalectomized muscle is based on the assumption that the same number of fibres are associated with the same dry weight of tissue. It will thus appear that there is a fall of 27 in the 'idiomolar' value and a rise of 29 in its electrostatic equivalent.

Computing η values in muscle from the direct analyses, and omitting anserine (the determination of which is not very exact), there appears a fall of about 9–10 mmol./kg. of fibre water due to the fall in total hexose esters and in Na over the small gains of phosphocreatine and magnesium. The remaining 17 mmol. would remain unaccounted for by the direct analyses.

Table 5. Concentrations in the 'fibre water'

	(Symbols as in text)	
	Normal	Adrenalectomized
k_1	152 m-equiv./l.	170 m-equiv./l.
l_1	4.7 m-equiv./l.	6.1 m-equiv./l.
[HCO ₃]	0.9 m-equiv./l.	1.2 m-equiv./l.
s	0.107	0.09
W	0.662	0.688
Dry wt.	0.232	0.226
V	1.00	1.07
η	147	120
ϵ	146	175

(V is calculated on the basis that the same number of fibres after adrenalectomy are associated with the same dry weight of tissue. Thus W above (the water content of the fibres/kg. muscle) for the adrenalectomized rat is then multiplied by $\frac{232}{226}$ and $V = \frac{0.688}{0.662} \times \frac{232}{226} = 1.07$.)

DISCUSSION

Inorganic changes. With regard to the inorganic constituents of the plasma, apart from the well-known changes in K and Na content, the mean increase in Mg is noteworthy and is accompanied by a similar increase in muscle Mg; the ratio of increase is similar to that of K. Total P and inorganic P appear also to be increased, with a fall in the bicarbonate and pH.

In muscle the mean changes observed by us for K, Na, P (total), Cl, and water follow closely those of Harrison & Darrow (1928). In addition we have shown a mean increase in Mg of 16–17%—practically the same relative increase as for the potassium. A marked increase in muscle Mg does not appear in the experiments of Buell & Turner (1941). This rise in muscle Mg may contribute to the asthenia after adrenalectomy, since it has been shown that Mg inhibits adenosinetriphosphatase (Greville & Lehmann, 1943).

Changes in η and ϵ . η , or the total non-diffusible substances falls from 147 to 120 after adrenalectomy, and ϵ , the electrostatic equivalent, rises from 146 to 175 when these values are determined by equations (1) and (2), and when V for adrenalectomized muscle is calculated on the assumption that a similar number of fibres are associated with the same dry weight of tissue. There is thus a fall in the total number of those characteristic molecules which the cell retains and which do not diffuse through its membrane. This is accompanied by a rise in the sum of their negative charges.

Computing η values in muscle from the direct analyses and omitting anserine, there appears a fall of about 9–10 mmol./kg. of fibre water due to the fall in total hexose esters and Na as against the small gains of phosphocreatine and magnesium. The remaining 17 mmol. would remain unaccounted for by the direct analyses.

The increase of ϵ , i.e. the increase in the negative charges on the non-diffusible molecules, must occur to accommodate the increase of K beyond what is accounted for by the decrease in Na.

The sodium change. Clearly any accurate assessment should be based on the same number of fibres, and using the dry weights, the same number is contained in 1.03 kg. of adrenalectomized muscle as in 1 kg. normal muscle. Now in 1 kg. normal muscle there are 27.1 mmol./Na, and in 1.03 kg. adrenalectomized muscle there are 20.3 mmol./Na. In the normal muscle s or the interspace is approximately 0.107 l. containing 0.107×138 mmol. = 14.8 mmol.; hence 27.1 - 14.8, or 12.3 mmol., are associated with the fibres. A similar calculation gives $20.3 - (0.087 \times 1.03 \times 128) = 6.9$ mmol. associated with the fibres in the adrenalectomized muscle. There is thus a fall of 12.3 - 6.9 = 5.4 mmol. It is obvious that the evidence for this change really rests on the somewhat uncertain ground that the same number of fibres are associated with the same total dry weight of muscle, but as it stands it amounts to a value of about 4% of the external Na. There is at the same time a gain of K which, disallowing for the relatively very small interspace K, amounts to $(118.5 \times 1.03) - 101 = 21$ mmol./kg. There is thus no correspondence between the K gain and the Na loss.

The loss of Na raises the question as to whether it is in fact extruded from within the fibre. The authors consider it more reasonable to suppose that it represents a small Na loss from an essentially extracellular source, which may be provisionally regarded as the sarcolemma.

The hexose esters. Of much interest are the changes in the concentrations of glucose-1-phosphate, glucose-6-phosphate and fructose-6-phosphate. Such changes are relatively very large. Glucose-1-phosphate is increased to 134% of the control value, and the other two decreased to 32% and 37%. We consider it preferable to defer an explanation of such changes to a later communication, with fuller studies of the carbohydrate changes, and further controls. At present, attention

may be called to the fact that the effect on the glucose-1-phosphate appears to relate to the findings of Schumann (1940) confirmed by Verzár & Montigel (1942) in which it was shown that phosphorylase action was inhibited in excised muscle from adrenalectomized rats as compared with similar muscle from normal rats.

SUMMARY

1. Skeletal muscle of albino rats (almost all of Wistar strain) was analyzed for various constituents 3-6 days after adrenalectomy, and compared with similar analyses on control rats, maintained on the same diet (containing low Na). The substances determined were K, Na, Mg, Cl, total P, acid-soluble P, phosphocreatine, adenosinetriphosphate, total hexose-monophosphate, glucose-6-phosphate, glucose-1-phosphate, fructose-6-phosphate, fructose-1:6-diphosphate, phosphoglyceric acid, phosphopyruvic acid, carnosine, anserine, water content. An increase after adrenalectomy was shown for K, Mg, phosphocreatine, glucose-1-phosphate. A decrease was shown for Na, glucose-6-phosphate, fructose-1:6-diphosphate.

2. Plasma constituents for the same rats, or rats under similar conditions were also determined. The substances or factors investigated were K, Na, Ca, Mg, pH, Cl, HCO_3^- , inorganic P, total P, urea, total molecular concentration, water content. Following adrenalectomy there was an increase of K, Mg, urea, total phosphate and inorganic phosphate. A decrease was shown for Na, Cl, HCO_3^- , Ca and the pH being probably decreased.

3. The η value, or total non-diffusible substances (reckoned as mmol./l. of 'fibre water'; Boyle & Conway, 1941) falls from 147 to 120 after adrenalectomy; the ϵ value (or mmol. alkali/l. of 'fibre water' required to satisfy the surplus of negative charges on η) rises from 146 to 175. Somewhat less than half the fall in η is accounted for by the direct analyses of the muscle constituents.

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The Nucleoprotein Content of Fibroblasts Growing *in vitro*

3. THE USE OF DEFATTED MEDIA

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It is generally accepted that lipid materials are inhibitory to the growth of tissues *in vitro* (cf. Baker & Carrel, 1925*a, b*; Mayer, 1936). We have already shown that the removal of fat from sheep embryo extracts increases the growth-promoting power of such extracts for tissue cultures of chick heart fibroblasts (Davidson & Waymouth, 1945). It has therefore seemed to us desirable that the effect on the growth of cultures of removal of the lipids from the embryo extract of the plasma coagulum or both, should be further investigated. In the course of this work we have prepared fat-free fowl plasma in the form of a sterile powder which can be stored indefinitely (Waymouth & Davidson, 1945). The use of a large batch of such plasma powder enables a long series of cultures to be set up in the same medium and avoids the necessity for the maintenance of a stock of liquid plasma which must be renewed frequently and which may vary in composition from batch to batch.

The use of defatted plasma has also enabled further investigations to be made on a factor in pancreatin which profoundly influences the morphology of fibroblast cultures *in vitro* (Davidson & Waymouth, 1944*a*). This factor appears to be identical with, or related to, the enzyme lecithinase A which hydrolyzes lecithin to lysolecithin. To examine the effect of this enzyme on growing tissue defatted media are obviously desirable.

This paper deals with the phospholipin content of embryonic tissues and of embryo tissue extracts before and after defatting, with the preparation of defatted fowl plasma and its chemical composition, and with the use of these defatted media in tissue culture technique.

METHODS AND MATERIALS

Tissue culture technique

(*a*) *General*. Cultures were grown by the roller tube technique of Willmer (1942) which we have previously employed (Davidson & Waymouth, 1943, 1945). Each roller tube contained 12 pieces of tissue in two rows of 6. As in earlier work all the cultures were made from fresh explants from the 10-day chick embryo heart and consisted therefore to a large extent of the cells which are usually termed 'fibroblasts' in tissue culture work (Mayer, 1939) but which have been more precisely defined as 'mechanocytes' by Willmer (1945). The amount of tissue present was determined by making estimations of nucleoprotein phosphorus (NPP) (Davidson & Waymouth, 1943).

(*b*) *Materials*. Lecithin was prepared from egg yolk by the method of Macfarlane & Knight (1941), and dissolved in ethanol. Insoluble material was centrifuged off and an emulsion prepared by adding 2 ml. of the ethanolic solution to 25 ml. of boiling 0.9% NaCl (Ponder, 1942). The solution was heated for 10 min. in a boiling water-bath to sterilize it and to drive off the ethanol. The concentration of this emulsion was estimated from the P content and suitable dilutions made with Tyrode solution.

Two samples of lysolecithin, of fractions 2 and 7 of King & Dolan (1933), were kindly presented by Prof. E. J. King. 5 mg. portions were dissolved in chloroform, reprecipitated with acetone and dissolved in 1.5 ml. of 0.9% NaCl. The solutions were sterilized by heating in a boiling water-bath at 100° for 10 min., cooled and diluted with Tyrode solution.

The pancreatin preparations were prepared according to the method previously described (Davidson & Waymouth, 1944*a*). They were dissolved in water, sterilized by heating to 100° for 10 min., cooled, and treated with an equal volume of double strength Tyrode solution. Similar preparations were also heated in alkaline solution (pH 8-10) in order to destroy such resistant enzymes as lecithinase A and