CXCII. CORTICO-ADRENAL INSUFFICIENCY AND POTASSIUM METABOLISM.

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I. DETERMINATION OF POTASSIUM IN SMALL QUANTITIES OF BLOOD AND TISSUES.

A RISE in blood potassium following adrenalectomy was first reported by Baumann & Kurland [1926], who found an average value of 28 mg./100 ml. in a series of 11 cats. Hastings & Compere [1931] found that serum potassium rose in dogs from an initial average concentration of 3 mM to one of up to 20 mM; these authors suggested that very similar values are encountered in potassium poisoning. Their paper does not, however, give any experimental details, such as the method for the determination of potassium or at which stage of adrenal insufficiency the samples were taken; moreover, the concentrations for normal dogs are distinctly lower than those reported by other authors. Kerr [1926], finds 4·7–5·6 mM, and the insufficiency values are higher than those reported by different workers. Zwemer & Sullivan [1934] reported an average value of 34·7 mg./100 ml. (22 determinations) with higher values in later insufficiency. In the human subject, Marañon *et al.* [1934] find maximum pre-mortal values of 49·5 mg./100 ml. with a mean value of 31·1 mg./100 ml. in Addisonians. Urechia *et al.* [1935] state that serum K of cats may rise to as much as 250 mg./100 ml. 10 min. after adrenalectomy (7 cats, survival time 15–64 hours). The very short survival time of their animals suggests that death supervened rather from operative damage than from adrenal insufficiency, whilst the high values reported are indicative of some methodical error, since we have found that the extreme values, obtained at death from cats poisoned with potassium, do not exceed 70 mg./100 ml. (unpublished work). Loeb *et al.* [1933] ascribed the high potassium values in dogs to haemolysis, with liberation of K from the erythrocytes; since, however, the K content of dog corpuscles is not significantly higher than that of the plasma [Kerr, 1926] it should rather be considered that a high plasma K is also a symptom of adrenal insufficiency in dogs.

Whilst high blood potassium has been found by most investigators in cortico-adrenal insufficiency, the actual levels found vary widely, and no systematic attempt has been made to correlate the symptoms with the blood potassium level. Further, the results are open to objection on several counts. Thus, Kerr [1926] has shown that the blood potassium of dogs may rise from 6·5 to 15 mM as a result of repeated haemorrhage. The amounts of blood taken for sampling by certain of the workers cited above were such as might be expected to lead to a similar result, as well as definitely to shorten the survival period.

In the case of human blood, haemolysis of even a small percentage of the erythrocytes might lead to a very high value for potassium. Whilst it is our opinion that Marañon's results are not due to this cause but represent the actual conditions in Addison's disease, yet it is felt that a reinvestigation of blood potassium values in human adrenal insufficiency is desirable.

In view of the above, the present reinvestigation of the behaviour of blood potassium during the course of experimental adrenal insufficiency in cats was undertaken. The research involved the elaboration of a method which would allow of the analysis of small quantities of peripheral blood; it was then possible to take daily samples without thereby significantly affecting the course of the syndrome.

Since the method involved taking whole blood, the most suitable experimental animals were cats, in which Abderhalden [1898] has shown that potassium is equally distributed between plasma and cells.

EXPERIMENTAL.

Known amounts of potassium (0.02–0.16 mg.) were determined by a colorimetric method based on that of Breh & Gaebler [1930] taking a standard containing 0.1 mg. K. The results are represented by curve 1, Fig. 1; the curve,

Fig. 1. All points represent the mean of 4 determinations.

being rectilinear, has the general equation $y = ax + b$, where x and y are respectively mg. K found and taken; by substituting different values of x and y , a is found to be 0.9, and b , 0.01; the final form of the equation is then

$$y = 0.9x + 0.01.$$

The same equation may be derived on the assumption that, under the given standard conditions, a constant loss of the precipitate takes place, irrespective of the actual amount present; if this be represented by k , the result of the colorimetric reading may be represented by

$$\frac{S - k}{y - k} = \frac{S}{x},$$

where S represents mg. of K in the standard, and y and x have the same significance as before. Substituting 0.1 for S , and different values for x and y , it will be found that $k = 0.01$, and substituting this value in the second equation, and simplifying, it again assumes the final form $y = 0.9x + 0.01$.

It can be concluded that accurate values will be given by methods of the type in question only when the sample under analysis has the same K content as the standard, and that increasingly divergent values will be obtained the greater the difference in K content. Further, these results are adequately explained on the assumption that a constant loss,¹ equal to 0.01 mg. K, takes place under the given conditions. The results obtained should therefore be corrected accordingly; a simpler procedure is to add 0.01 mg. K to all the tubes, and this was adopted in the method described below. Curve II, Fig. 1, shows the results obtained for the determination of 0.02–0.18 mg. K; it will be seen that values little different from theoretical are obtained over the whole range studied.

Special reagents.

A. Standard K_2SO_4 solution containing 2 mg. K per 100 ml.

B. Precipitating reagent: 210 ml. of a solution of 120 g. of K-free $NaNO_2$ in 180 ml. of water are added to a solution of 25 g. of $Co(NO_3)_2$ in 50 ml. of water and 12.5 ml. of glacial acetic acid, and air is bubbled through the solution for 1–2 hours to complete elimination of NO_2 . The solution should be kept at 0° and re-aerated weekly; after a month it is advisable to reject it. When taken for use, the clear liquid is decanted off, a tenth of the volume of 40% $AgNO_3$ is added, and the solution is shaken and filtered.

C. 0.5% sulphanilic acid in 30% CH_3COOH .

D. 0.5% α -naphthylamine in 30% CH_3COOH .

In the actual determination of K in blood, duplicate 0.2 ml. samples of whole blood were taken in oxalated blood-pipettes from an ear vein, rejecting the first drop appearing after making the incision. They were immediately diluted in 5.9 ml. of water in a 15 ml. conical pyrex centrifuge-tube. 0.4 ml. each of 10% sodium tungstate and $2/3 N H_2SO_4$ were added, the mixture was shaken, 0.1 ml. of 5% $AgNO_3$ was added, the tubes were again shaken and centrifuged (3000–4000 r.p.m.; 15 min.). 5 ml. of each centrifugate were transferred to 15 ml. graduated centrifuge-tubes, the tubes were warmed to 60–70°, and 0.2 ml. each of reagent B were added, as well as to two tubes containing 5 ml. of standard K_2SO_4 solution (solution A); 0.5 ml. of solution A had previously been added to all tubes. Two hours later the tubes were centrifuged, the supernatant fluid was siphoned off (leaving 0.2 ml.), and the precipitate of K argenticobaltinitrite was washed 3 times on the centrifuge with three 7 ml. portions of water. 5 ml. of 0.2 N NaOH were then added to the precipitate in each tube, the suspension was boiled to break down the complex, cooled, diluted to exactly 6 ml. in each tube and centrifuged. 1 ml. of each of the centrifugates was transferred to 50-ml. volumetric flasks containing 15 ml. of 10% acetic acid, 2 ml. of sulphanilic acid and 1 ml. of α -naphthylamine solutions were added to the flasks, which were then filled to the mark with 10% acetic acid and allowed to stand for 10 min., when the colours were compared in a Duboscq type colorimeter, using a blue filter. The potassium content of the blood is given by

$$\text{mg. K/100 ml.} = \frac{S \times K_s \times 100}{R \times V},$$

where S is the level at which the standard is set (usually 20), K_s is the amount of K actually taken for the reading (0.1/6 mg.), R is the reading of

¹ This loss probably represents the difference between loss due to solubility of K argenticobaltinitrite, and a gain due to presence of minute amounts of K in the reagents. For these reasons it will be obvious that k should be determined empirically in each laboratory, and that it should be redetermined should any of the reagents be procured from a new source.

the unknown, and V is the volume of the blood corresponding with the amount of NaOH solution finally taken; should this be 2 ml., $V = 0.2 \times 5/7 \times 2/6$. Hence mg. K/100 ml. is in the given case equal to $70S/2R$.

If the sample under analysis contains <0.05 mg. of K, it will be desirable to take 2 ml. or more of the final centrifugate, but if its K content is >0.2 mg., it will not be possible to compare the colour with that of the standard, owing to formation of a flocculent precipitate; in such cases, the amount of final centrifugate taken should be reduced to 0.5 ml.

Presence of Ag in the blood centrifugate may lead to considerable error in the determination, as under the given conditions sparingly soluble, colourless needles of AgNO_2 tend to separate out together with the precipitate of K argentocobaltinitrite. These crystals will, if present in small amount only, be eliminated during the process of washing; otherwise part of them remains and leads to the obtaining of fictitiously high values for K. In normal blood, the amount specified (0.1 ml. of 5% AgNO_3) is slightly less than that required for complete precipitation of Cl^- ; in the hypochloreaemia sometimes encountered in advanced adrenal insufficiency it is probable that some small excess of AgNO_3 remains, but this has not been observed to interfere. If, however, the laboratory temperature falls below 15° during precipitation, the crystals will often separate in considerable quantity; this is avoided by maintaining the temperature at about 20° .

A further source of error lies in the fact that NH_4^+ is precipitated together with K; with the small amounts of ammonia present in freshly drawn blood this will not significantly affect the results, but where the sample has been allowed to stand at room temperature for any length of time considerable error may result. It is therefore advisable to deproteinize as soon as possible after collection of the sample and to keep the centrifugates in the ice-box should precipitation of K not be effected immediately.

With the given amount of blood, deproteinization was found to be incomplete when less than the specified amount of the appropriate reagents was taken.

In late adrenal insufficiency the peripheral circulation is highly inadequate, and at this stage it is often necessary to take blood from the heart. In such cases, and for plasma or serum, the following procedure has been applied; 0.5 ml. of the sample is diluted to 7.7 ml., 1.0 ml. each of tungstate and sulphuric acid solutions and 0.3 ml. of 5% AgNO_3 are added, and 5 ml. of the centrifugate are taken for the determination.

Application to determination of potassium in muscle.

0.1–0.3 g. of skeletal or cardiac muscle is weighed in a tared, graduated pyrex centrifuge-tube, and subjected to wet incineration, according to the method of Leulier & Bernard [1934] (heating on a gauze with 0.5 ml. of a mixture of 35 ml. of HClO_4 and 65 ml. of H_2SO_4); the heating is continued to almost complete elimination of HCl, and the colourless solution is then diluted with 2 ml. of water and made neutral by adding a small excess of Li_2CO_3 . The excess of the latter is dissolved by adding a few drops of glacial CH_3COOH , 2 ml. of 20% NaNO_2 and 0.5 ml. of glacial CH_3COOH are added, and the tube is heated at 100° for 30 min. 0.1–0.3 ml. of 5% AgNO_3 is then added, the solution diluted to 10 ml. and 1 ml. transferred to a second tube containing 4 ml. of water. K is then determined by the method described above for blood, when, if equal volumes of the final centrifugates are taken, mg. K/100 ml. is given by $100 S/WR$, where W is the weight of tissue taken.

The above procedure is applicable to any other tissue. Where only very small amounts of tissue are available, the steps of dilution and transference of an aliquot part of the solution could be eliminated; the amount of tissue taken for incineration should not be less than 5–10 mg., containing 0.02–0.04 mg. K.

Speed and accuracy of the method.

The time required to perform 7 duplicate determinations of blood potassium has been found not to exceed 7–8 hours, if the operations of siphoning off the liquid and washing the precipitate in 8 tubes are performed while the remaining tubes are centrifuged.

No very high degree of accuracy is claimed, as the results obtained in parallel determinations often vary by $\pm 5\%$ from the mean value.

II. BLOOD POTASSIUM OF NORMAL AND ADRENALECTOMIZED CATS.

A. Normal cats.

Abderhalden [1898] gives the K content of normal cat blood as being 21.6 mg. per 100 g., in serum, whole blood and cells. Baumann & Kurland [1926] report an average value of 19.0 mg. per 100 ml. (varying from 15.9 to 24.4) of plasma, and 24.1 (19.7–28.8) mg. per 100 ml. for whole blood. D'Silva [1934] finds that the K content of serum varies from 16 to 22 mg./100 ml. and the values reported by Zwemer and Sullivan¹ for normal cat serum (15–22 mg./100 ml.) likewise fall within very similar limits.

In a series of 19 normal cats (males, 3.5–4 kg. in weight) a mean value of 19.3 (14–23.1) mg./100 ml. of K was found for plasma, using the methods described in Part I. The same animals had values of 20.2 (16.2–26.1) mg./100 ml. for serum, and 21.4 (16.0–26.1) mg./100 ml. for whole blood. These values are substantially identical with those of previous workers, and confirm the equal distribution of K in the cellular and non-cellular constituents of the blood of cats.

B. Adrenalectomized cats.

The adrenal glands were removed under ether anaesthesia, and under conditions of strict asepsis, either by a two-stage operation (dorso-lumbar incision), allowing 1–3 weeks to elapse between the first and the second operations, or by a single operation (mid-abdominal incision), applying a new technique described elsewhere. The survival periods were similar with either procedure so that the single operation was accordingly performed in most cases. The cats recovered rapidly from the operation, as far as could be judged from their appetite and activity; the animals were allowed tinned salmon and milk *ad lib.*, and in general no marked anorexia was evident earlier than the fifth day after the operation. Care was taken to maintain the laboratory temperature as far as possible constant (about 20°), in view of the known sensitivity of adrenalectomized animals to temperature fluctuations. The average survival time was 10 days, and blood was taken at intervals after the operation.

The results are given in Table I, from which it appears that the average blood K rises during the first 3 days following adrenalectomy, to a maximum value of

¹ The figures in this paper are erroneously given as mg. K/100 ml.; they should be mg. KCl/100 ml.

Table I. *Blood potassium in adrenal insufficiency.*

Each value is the mean (mg./100 ml.) of two concordant determinations, on whole blood, plasma or serum.

Cat no.	Days following bilateral adrenalectomy														Survival in days
	0	1	2	3	4	5	6	7	8	9	10	11	12	>12	
3623	22.2	27.3	—	33.3	29.4	—	—	—	—	—	—	—	—	—	5
3504	17.0	—	—	—	20.5	21.7	27.5	—	—	—	—	—	—	—	6
3509	21.0	—	—	—	—	—	27.6	—	—	—	—	—	—	—	6
3503	20.0	—	—	—	—	—	22.4	—	—	—	—	—	—	—	7
3605	20.0	—	—	41.0	—	33.5	—	—	—	—	—	—	—	—	7
3601	—	—	—	—	24.6	—	44.4	—	44.4	—	—	—	—	—	8
3506	—	—	—	—	—	—	23.2	—	—	18.3	—	—	—	—	9
3512	21.3	—	—	—	—	—	31.1	—	—	31.2	32.0	—	—	—	10
3508	20.0	—	—	—	—	—	—	—	22.5	—	24.2	—	—	—	11
3627	22.1	25.2	28.5	37.8	35.0	22.3	20.5	21.0	11.8	11.1	17.1	17.5	22.7	45.7	13
3624	24.2	22.2	—	31.2	39.3	27.1	29.6	31.1	32.2	25.7	23.5	24.8	22.8	26.3 25.2 22.1	15
3510	20.0	—	—	—	30.6	—	—	21.5	33.3	—	—	—	—	27.5 35.0	16
3602	—	—	—	42.5	—	44.2	—	—	—	39.0	—	31.6	—	35.1 31.3 41.5	17
3603	17.5	—	—	—	—	36.3	—	—	32.2	—	34.2	—	37.5	46.1 31.0	31*
3611	—	—	21.4	—	—	26.9	30.2	—	—	—	—	—	—	—	†
3621	—	—	25.3	32.8	—	—	—	—	—	—	—	—	—	—	†
3628	16.0	—	28.0	—	—	—	—	—	—	—	—	—	—	—	†
3629	—	—	30.2	—	—	—	—	—	—	—	—	—	—	—	†
Average	20.1	24.9	26.7	36.4	30.0	30.3	27.9	24.5	29.4	25.1	26.2	24.6	27.7	34.2	—
No. of deter- minations	12	3	5	6	6	7	9	3	6	5	5	3	3	11	—

* Used for other experiments.

† Probably had accessory tissue.

36.4 mg./100 ml., thereafter fluctuating. Considerable variations are found during the course of the syndrome, depending on the stage of insufficiency and the individual behaviour of the animals. Blood K substantially identical with normal may be encountered and, unless daily samples are taken, particularly during the first 3-5 days after adrenalectomy, the higher values may be missed. Cats 3624 and 3627 drank considerable amounts of water after the fifth day following adrenalectomy, and this, together with their restricted food intake, might explain the low values found subsequently.

The average value for the entire group agrees most closely with that of Baumann & Kurland [1926], but is also little different from that given by Zwemer & Sullivan. No values even approaching the maxima recorded by Hastings and Compere or by Urechia *et al.* have been found.

The values given in Table I represent results obtained for whole blood, plasma or serum. Although satisfactory evidence is available that in normal cats identical results are obtained for cells and plasma, yet the possibility remained that the rise in whole blood might apply only to the plasma, or to the cells. In

Table II. *Distribution of potassium between cells and plasma in adrenalectomized cats.*

Each value is the mean of two determinations.

Cat no.	Days after adrenalectomy	mg. K/100 ml. in		
		Cells	Plasma	Whole blood
3504	6	21.3	21.7	17.7
3504	7	—	27.5	30.5
3506	10	—	18.3	15.6
3510	16	36.2	37.5	37.8

order to exclude this objection, potassium was in some cases determined separately for whole blood, plasma and cells (Table II); it is evidence that the distribution is uniform in the cases studied.

III. POTASSIUM CONTENT OF SKELETAL AND CARDIAC MUSCLE IN CORTICO-ADRENAL INSUFFICIENCY.

Samples of muscle were taken from the adductor femoris and from the heart of animals killed when in the terminal stage of insufficiency, or immediately after death, and K was determined by the method described above.

Table III. *Potassium content of muscle.*

Each value is the mean of two determinations.

Cat no.	Days after adrenalectomy	Dry content of muscle	mg. K/100 g. in	
			Wet muscle	Dry muscle
Skeletal muscle.				
Normal	—	28.2	501.6	1778
—	—	27.2	459.2	1689
Adrenalectomized.				
3609	8	22.4	437.7	1954
3615	9	20.6	408.1	1981
3627	12	21.1	382.0	1811
3624	15	20.2	375.4	1858
3602	17	22.8	431.5	1893
3603*	31	24.1	456.7	1895
Average		21.9	415.2	1900
Cardiac muscle.				
3615	9	21.9	305.8	1396
3627	12	22.0	309.0	1405
3624	15	20.5	211.2	1030
Average		21.5	275.3	1277

* Presence of accessory cortical tissue probable.

The results given in Table III indicate that the K content of the fresh muscle is lower than in normal animals (the literature gives values of 450–500 mg./100 g.). At the same time, the water content is higher, so that the results calculated per 100 g. of dry muscle are actually higher than for normal cats.

Leulier *et al.* [1933] give the potassium content of normal cat myocardium as 365–368 mg./100 g. the values found in insufficiency will be seen to be significantly lower.

DISCUSSION.

It may be concluded on the basis of the above evidence that, in cats, bilateral adrenalectomy is followed by a rise in blood K; this in most cases attains its maximum on the third or fourth day after the operation, thereafter falling to a more or less constant level of 30 mg./100 ml. average, which is also the level most often encountered at death. In individual cases normal and subnormal values may be encountered in the period following the initial rise.

The high K characteristic of cortico-adrenal insufficiency may be due to disturbances in the renal excretory function, to liberation from or lack of fixation in the tissues, or to both of these causes. Were the kidneys alone to be at fault, it might be expected that the rise in plasma K would be associated with a rise in tissue K; actually the results obtained for muscle indicate a subnormal value. On the other hand, there is no reason to believe that a normally functioning kidney could not deal adequately with the not very great amounts of K liberated from the tissues. It would hence appear to be most probable that, whilst both the causes mentioned may take effect simultaneously, the more important would be inability to eliminate K (exogenous or endogenous) entering the blood stream.

From this viewpoint, the variations in blood K following adrenalectomy may be interpreted in the following way. Anorexia does not appear until the third or fourth day after the operation; this coincides with the first high level. Up to this point, K of alimentary origin has been undergoing resorption and has tended to accumulate in the blood stream. Following this, the animals on an unvaried diet tend more and more to limit their food intake and to drink water; at the same time progressively lower values are found for blood K. The activity of the cats varies parallel with their appetite, and since muscular activity is associated with passage of K from the muscle cells to the plasma, this would function as a second factor, tending to introduce K into the blood stream during the first period of rising K and to withhold it during the succeeding stage. The final rise in blood K is ascribable to tissue breakdown, due to inanition, and to progressive aggravation of the kidney lesions.

It has been suggested by Hastings and Compere and by Zwemer and Sullivan that the higher values for blood K found by them are of the same order as those found in animals dying after intravenous administration of a lethal dose of KCl. Marañón *et al.* ascribed the symptoms of Addison's disease to defective regulation of water metabolism, due in turn to the high blood K, and Urechia *et al.* stated that the muscular debility of adrenal insufficiency might be due to K poisoning.

The most striking symptoms encountered in cortico-adrenal insufficiency are anorexia, muscular weakness, low blood sugar, low blood Na, high N.P.N., degenerative changes in the proximal renal tubules, vascular disturbances involving stagnation of the peripheral circulation and anhydraemia. At death, the heart is invariably found in diastole, often with considerable dilation. Experiments performed by the present authors on normal and adrenalectomized cats [Zwemer & Truszkowski, 1936] have shown that all the symptoms enumerated may be reproduced in normal animals when the blood K level is maintained over comparable periods at the levels (30–40 mg./100 ml.) found in adrenal insufficiency. In adrenal insufficiency the ability to dispose of injected K salts is diminished so that doses not significantly raising the blood K of normal animals are followed by a considerable, prolonged and often fatal rise.

SUMMARY.

1. A method for the determination of K in 0.2 ml. of blood has been described, and certain errors present in the ordinarily used methods pointed out.
2. The normal values found (19 cats) were: plasma 19.3, serum 20.2 and whole blood 21.4 mg. K/100 ml.
3. The results of analyses performed on 18 bilaterally adrenalectomized cats, at frequent intervals following the operation, are given; the values for blood K

vary within wide limits (11–46 mg./100 ml.) for different animals at different times after the operation. The most consistent effect is that of a rapid rise to a maximum value on the third or fourth day.

4. A method for determining K in muscle is described: an average of 0.415 % wet weight, or 1.900 % dry weight has been found for skeletal muscle of animals in the terminal stage of adrenal insufficiency, and of 275 mg./100 g. and 1.277/100 g. for myocardium. The values for wet weight are lower, and for dry weight higher, than for normal cats.

5. The significance of these findings is discussed, and it is suggested that the syndrome of cortico-adrenal insufficiency is intimately associated with a disturbance in K metabolism.

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