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THESIS

on

Research Work carried out in the Biochemical

and Physiological Laboratories of the

University of Manitoba

under the direction of Prof. A. T. Cameron

- I. The Nature of Chlorine Combination in Urine.
- II. The Effects of Inanition upon the Adrenal Bodies
and other Organs.
- III. The Action of Various Anions on the Frog's Heart
and Muscle-Nerve Preparations.

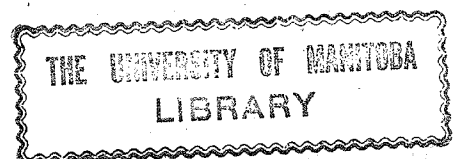
Submitted to the University of Manitoba

for the Master of Arts Degree.

April, 1921

M. S. Hollenberg.

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THE NATURE OF CHLORINE COMBINATION IN URINE

Neuberg⁽¹⁾ states that Berlioz and Lepinois⁽²⁾, and other French writers found chlorine present in urine partly in organic combination. Later investigators^(3,4) denied this. Baumgarten⁽⁵⁾ obtained results indicating that 0.04 to 0.2 gm. of organic chlorine is excreted daily in the urine; the maximum found amounted to about 10 per cent of the total chlorine excretion, and was independent of the amount of sodium chloride in the diet and the temperature of the individual. We have been unable to find any more recent work dealing with the possible presence of organic chlorine in urine.

Baumgarten's method consisted in treating urine with sodium nitrite and fuming nitric acid (to reduce chlorates), adding excess of silver nitrate and filtering in darkness, and, after removal of excess silver, concentrating to one-third, again filtering, and heating the filtrate in a retort with concentrated nitric and sulfuric acids free from chloride. The distillate was collected in a dilute silver nitrate solution, and silver chloride was precipitated in this corresponding to the amounts of chlorine indicated above. This chlorine was considered to be in organic combination.

We have repeated Baumgarten's procedure with completely negative results.

Subject No.	Chloride-chlorine in 100 cc. urine	Chlorine and oxy-chlorine in 100 cc. urine
1.	0.7837 gm.	0.7837 gm.
2.	0.6226	0.6225
3.	0.5646	0.5649
	0.6220	0.6225

TABLE I

Chlorates and perchlorates do not appear to occur normally in urine, and when administered are excreted in the urine unchanged and almost quantitatively within 48 hours. (6) We have confirmed the absence of chlorine-oxy-acids from normal urine by the following procedure.

The halide content of 20 cc. of urine was precipitated as silver chloride in the presence of nitric acid, the precipitate was collected on a Gooch crucible, washed with distilled water, and dried at 100° C. to constant weight. Another 20 cc. of urine was treated with nitrite and fuming nitric acid, allowed to stand for some hours, and then the halide content estimated as just described. The results indicate that chlorine oxy-acids can be considered as absent from normal urine (Table I)

We have made a careful comparison of the chloride (halide) content and the total chlorine (halogen) content of urine. The halide in 20 cc. of urine was estimated as usual. The total halogen was estimated by evaporating 20 cc. of urine with 10 gm. of solid sodium hydroxide, heating the fused mass with additions of very small quantities of potassium nitrate until all organic matter was oxidized, dissolving in water, and estimating the halide as silver chloride as usual. The results are given in Table II.

The slightly greater differences with the pathological urines were probably due to difficultly filterable organized matter, retained in the Gooch crucible but destroyed during fusion. The close agreement indicates the absence of organic chlorine.

TABLE II

<u>Subject No.</u>	<u>Condition</u>	<u>Chloride-chlorine in 100 cc. urine</u>	<u>Total chlorine in 100 cc. urine</u>
1.#	Normal	1.0835 gm.	1.0834
		1.0836	1.0831
		1.0831	1.0834
2.	"	0.6227	0.6235
		0.6216	0.6232
4.	"	0.9383	0.9365
		0.9358	0.9387
5.	"	0.9451	0.9449
		0.9447	0.9444

TABLE II (Cont'd)

<u>Subject No.</u>	<u>Condition</u>	<u>Chloride-chlorine in 100 cc. urine</u>	<u>Total chlorine in 100 cc. urine</u>
6.	Tubercular	0.3034 gm. 0.3041	0.3028 gm. 0.3037
7.	"	0.4876 0.4880	0.4867 0.4864
8.	"	0.4480 0.4477	0.4474 0.4476
9.	"	0.3670	0.3637

A fresh sample

All our figures are for halogens and halides (estimated as chloride). As under normal conditions only traces of bromides and iodides are present in urine, the results can be considered in terms of chlorine and chloride.

We conclude that in the absence of chlorate or similar medication chlorine occurs in urine as chloride only; organic chlorine and chlorine oxy-acids are absent.

B I B L I O G R A P H Y

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- (4) Meilliere, G., Compt. rend. Soc. biol., 1901, 1111, 1174.
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- (6) Heffter, A., Ergebn. Physiol., 1te Abt., 1903, 11, 108.

THESIS ON RESEARCH

CARRIED OUT IN THE BIOCHEMICAL AND PHYSIOLOGICAL

LABORATORIES OF THE UNIVERSITY OF MANITOBA.

- I. The effects of Inanition upon the Adrenal Bodies and other organs.
- II. The effects of different Anions upon Irritability and enzyme Action.

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The animals were killed by prolonged anaesthesia under chloroform. The different organs were dissected out carefully and immediately transferred to a glass container fitted with a ground glass stopper. The containers and organs were

METHOD OF INVESTIGATION AND RESULTS.

Although the cardinal object in this investigation was to determine the effect of inanition upon the adrenal bodies I also took the opportunity to investigate the effect of inanition upon some of the other important organs.

is a marked diminution in the adrenin content of these glands. In the adrenin content whilst during the later stages there stages of inanition of rats and dogs there is an increase of my experiments further indicate that during the early a distinct hypertrophy of the adrenal bodies. The results observations. After a period of inanition there is always experiments upon rats and dogs and am able to confirm his experiment upon pigeons and have also carried out similar takes place in guinea pigs. I have repeated McCarrison's adrenal glands. He later showed that this hypertrophy also inanition gives rise to a remarkable enlargement of the important discovery made by McCarrison (1) that in pigeons This investigation was undertaken as a result of the

INTRODUCTION.

AND OTHER ORGANS.

THE EFFECTS OF INANITION UPON THE ADRENAL BODIES

weighed to the fourth place on a chemical balance and the weight found by difference. The weights of the organs were then calculated as the percentage of the total body weight. I succeeded in confirming fully McCarrison's² observations with reference to the adrenals in pigeons. (Tables I, A I. B.) I have also shown that the adrenals of dogs and rats which were subjected to starvation also hypertrophy. (Tables 2A, 2B, 3A, 3B.)

The next part of my investigation was to determine whether it is the cortex or the chromophil tissue that hypertrophies during inanition. The first method I employed to determine whether or not an increase in chromophil tissue had occurred was 'the physiological test'.

An adrenal was taken from a normal dog and one from a starved dog. Both were weighed and an extract of each made. Saline solution was then added to each in proportion to its weight. The relative strengths of the two extracts were then compared by intravenous injection of equal quantities into a dog. The corresponding rise of blood pressure indicating the relative strengths of the adrenin content of the glands.

The above 'physiological test' was carried out with the glands of two inanition dogs - one of these was starved for fourteen days and the other for thirty days.

The results of these two tests showed that the animal