

THE METABOLISM OF THE NUCLEINS UNDER
PHYSIOLOGICAL AND PATHOLOGICAL CON-
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OUR knowledge of the changes which the nucleins undergo in the animal organism is still very far from being complete. What we have learned as to the changes which they undergo in and outside the body, we owe very largely to Kossel and those who have worked under him, although great advances were also made by Miescher, Altmann and others. The nucleins are obtainable from most nuclear-holding cells, although not from all. In the leucocytes, at least after death, they are present in the form of a compound with an albumose-like body, histon, viz., nucleo-histon. Miescher investigated the nuclein found in pus cells, Hammarsten that present in the pancreas, Halliburton and others similar bodies in different organs. They are usually combined with more or less detachable albumin, forming nucleo-proteids. It is with the changes which the nuclein radicle undergoes that we have to deal in the present paper. This radicle is made up of a more or less firm combination between an organic phosphorus-holding acid—nucleic acid—and albumin. It can as a rule be split up into its constituents by means of alkalies, pancreatic digestion and some other agents. From nucleic acid on hydrolysis with weak acids bases which contain the alloxan nucleus are easily obtained, and a base called cytosin, the constitution of which is not yet known, while all the phosphorus of the original acid still remains combined entirely in organic form as a substance to which the name of thymic acid has been given by Kossel. This decomposition takes place with the greatest ease, and the important points connected with the action are that no inorganic phosphoric acid appears and that no bases containing the alloxan nucleus are obtainable from thymic acid.

After the action of more concentrated acid solutions or after weaker solutions have acted for longer periods or at higher temperatures, the phosphorus gradually is split off in the form of orthophosphoric acid.

The nitrogen holding part of the thymic acid appears in different forms of combination, the end product being, of course, ammonia.

Now there are two of these decomposition products which we wish to draw special attention to as more or less characteristic signs of the decomposition of the nucleins, viz., the alloxur bases and phosphoric acid.

As a sign of the decomposition of nucleins in the organism, the bases are of the greatest importance, but too much stress must not be laid on the connection because, in the first place, in the body these alloxur bases in all probability undergo easily further alterations, and secondly they may be partially replaced at least by an acid containing the same nucleus, viz., uric acid. The conditions which exist in the organism leading to the derivation from the same mother substance of alloxur bases or uric acid respectively are still practically unknown. The experiments of Horbaczewski¹ although exceedingly valuable must be interpreted with the greatest caution because the conditions under which they were carried out are not such as exist in the living organism.

When we refer to the derivation of uric acid from the nucleins, we speak of a change which has not yet been shown to take place outside the body except in the case of Horbaczewski's experiments. Uric acid has not yet been directly obtained from the alloxur bases on simple oxidation by chemical means.

Within recent years a mass of literature, very largely clinical in nature, has accumulated on the relationship between leucocytosis or leucolysis and the excretion of the alloxur bodies (uric acid and the bases) in the urine.

The conclusions which a number of the writers have drawn from their work are hardly warrantable, either because the methods they employed were not free from errors or because the quantities of urine which they took for the estimation of, for example, the alloxur bases were far too small. It is too much to expect that under all conditions, physiological as well as pathological, with every increase in white blood corpuscles or with every hypoleucocytosis after a hyperleucocytosis

¹ *Monatshefte für Chemie*, x. S. 624, 1889; xii. S. 221-275. *Sitzungsb. d. Wiener Akad.* 1891, iii. S. 78, 132. *Zur Theorie der Harnsäurebildung im Säugethierorganismus*, 1892, J. F. Bergmann, Wiesbaden.

there should be a corresponding increase in the excretion of the alloxur bodies. We cannot compare such absolutely diverse pathological conditions as the leucocytoses of pneumonia, plumbism, carcinoma, leucocythæmia, or still more the experimental hyperleucocytoses met with after the injection of certain substances such as nucleic acid, the albumoses, etc., and we ought not to be surprised to find that there is not, with every rise in the leucocytes, a corresponding increase in the excretion of these alloxur bodies.

If we look at the experimental leucocytoses alone we do not yet know whether there be an increased relative or absolute leucolysis. There is much to be said for the chemotactic theory of Goldscheider and Jakob¹, and we must be ready to grant that under some conditions the leucocytosis may be simply one due to altered distribution of these cells. It is exceedingly difficult, if not impossible, to find out by histological methods whether certain leucocytes be undergoing decomposition or not. The changes which Gumprecht² has described as occurring in the nucleus of the leucocyte when it is undergoing degeneration, viz., the general swelling, gradual breaking down and final removal of the chromatic portion have not been detected by many others.

When the subject is examined from the chemical standpoint, one must be exceedingly careful in not at first drawing very important conclusions from the results of analyses which may be explained in a large number of different ways.

One must remember that, even when the nucleins are breaking down to a very marked extent, there is the possibility that the alloxur bodies may hardly be increased at all because the organism may still be able to transform them partly into urea.

We think then, bearing in mind that the excretion of the alloxur bodies must from their nature be a variable one, that it is most important to know in what way the phosphoric acid excretion is altered absolutely, and relatively to the total amount of nitrogen excreted, and also to find out the ratio that exists between the total nitrogen, nitrogen of the alloxur bodies and phosphoric acid in the urine under different conditions where alterations in the number and nature of leucocytes exist.

¹ *Zeit. f. klin. Med.* xxv. S. 373.

² *Verh. d. Cong. f. inner. Med.* 1896, S. 314; vide also E. Botkin, *Virchow's Archiv*, cxli. S. 238, 1895; cxlv. S. 369, 1896.

Before speaking of the work which we have done along the above-mentioned lines, we think it advisable to describe in the first place what is known as to the metabolism of the nucleins under physiological conditions.

When nucleins are taken by the mouth, the first change that they undergo in the alimentary tract is a simple solvent one in the stomach, and that only to a very slight degree. They are never split up into their constituents. They are easily broken up however by the pancreatic secretion into an organic phosphorus-holding acid (not nucleic acid) and albumose or peptone¹. The important points to notice are that the phosphorus is still in organic combination and that neither ortho- nor meta-phosphoric acid is so formed.

It is very probable that the organic phosphorus-holding acid so formed is similar to thymic acid. It forms soluble compounds with albumose and peptone and is, in all probability, so absorbed. After absorption the bodies derived from the nucleins cause a well-marked leucocytosis and the excretion of phosphoric acid in the urine is increased. Whether a hypoleucocytosis always precedes the hyperleucocytosis is difficult to say. Almost all the writers on this subject have emphasised the fact that, on giving nucleins by the mouth, the phosphoric acid excretion in the urine is increased; but they have omitted to show that this excretion cannot be accounted for by the phosphorus taken in the form of nucleins there being really more phosphorus excreted by the kidneys than was present in the original nucleins. Stadthagen² and Gumlich³ both showed this marked effect on the phosphoric acid excretion without drawing attention to its special importance. In the next place, with regard to the effect on the excretion of the alloxur bodies, there has been more or less controversy. Both Stadthagen and Gumlich were unable to notice any effect on the amounts of these bodies excreted, but later on Horbaczewski⁴, Weintraud⁵ and others said that, in giving large quantities of nucleins or food stuffs containing large quantities of them, there was a rise in the alloxuric nitrogen.

¹ Popoff. *Zeit. f. physiol. Chemie*, XVIII. S. 533, 1894. Milroy, *ibid.* XXII. S. 307, 1896-7.

² Stadthagen. *Virchow's Archiv*, CIX. S. 390. 1887.

³ Gumlich. *Zeit. f. phys. Chem.* XVIII. S. 508. 1894.

⁴ Horbaczewski. *Loc. cit.*

⁵ Weintraud. *Archiv f. Anat. u. Physiol. (Physiol. Abteil)* 1895, S. 382; *Verhand. d. 14 Congress f. inner. Med.* 1896, S. 190.

Now great caution has to be exercised in the estimation of the alloxur bases. At least 700 c.c. of urine must be used in order to get reliable results. Horbaczewski (*loc. cit.*) in a series of valuable experiments showed that the uric acid as well as the bases was increased after giving nucleins by the mouth. He points out also that this increase may be due to the resultant leucocytosis leading to a rise in the amount of alloxur bodies excreted. Horbaczewski emphasises the fact that with every rise in the leucocytes there is a corresponding increase in the amount of uric acid excreted; but even if this were the case, which is more than doubtful, we cannot at once jump to the conclusion that the sole reason for this is that the absolute amount of the breaking down of the leucocytes has been increased. The phosphoric acid formed by the oxidation of the phosphorus-holding part in the tissues may act as a depressor of the oxidative power of those tissues by the tendency that it has to neutralise part of the alkali of the blood. Again, it may be due to an altered metabolism in the leucocytes under the new conditions.

To prove that the rise in uric acid or alloxur bases or both is due to an absolute or relative increase in the normal leucolysis, one must show a corresponding rise in the phosphoric acid excretion and this is not always apparent. It is hardly likely that there is a marked retention of the phosphoric acid in the organism, and if there be not, then the absence of the rise in the phosphorus excretion negatives the view that the alloxur bodies are derived from the direct breaking down of the nucleins.

There is something to be said in favour of Mares¹ view that the uric acid is not necessarily a product derived from breaking down of the cell nuclei but may be derived from an altered metabolism in the cell. Horbaczewski constantly refers to the condition in leucocythæmia as if it were a similar one to the experimental leucocytoses. As we shall show at the close of the paper this is not so. The relationship which exists between the number of the leucocytes, the excretion of the alloxur bodies and the excretion of phosphoric acid in leucocythæmia is entirely different from that in the experimental forms of hyperleucocytosis.

We shall now describe the work which we have been occupied with during the last two years, and shall, in the first place, discuss the metabolism of the nucleins or tissues containing nucleins when these

¹ Mares. *Monatshefte f. Chemie*, XIII. pp. 101-110.

are taken by the mouth. As this has been worked at more or less fully by many people, we shall restrict ourselves to those points which we regard as of special importance. From what has already been said, it can easily be understood that the questions which particularly require to be answered relate to the ratios existing between the total nitrogen, nitrogen of alloxur bodies and phosphoric acid excreted in the urine.

When nucleins are given by the mouth, they undergo the changes which have been already described and act as substances which increase the number of white blood corpuscles. Now the one important question is, does this increase in leucocytes simply mean a redistribution of those cells or a withdrawal of them from their places of formation and after the effects of the absorbed products of nuclein digestion have passed off, are they simply redistributed afresh? That is to say, is the action simply a chemotactic one? Or is there with the hyperleucocytosis an increase in the breaking down of the leucocytes either accompanying or subsequent to their increase?

Now although Horbaczewski and others have described a rise in the alloxuric excretion along with the leucocytic rise, a number of experiments by others have not agreed with this view¹.

What we set ourselves especially to try to solve was the nature of the alterations quantitative and qualitative in the nitrogen and phosphorus metabolism.

The first set of experiments deal with the metabolism of the nucleins when given in the form of thymus tabloids.

The second and the third sets deal with the metabolism of nucleic acid and metaphosphoric acid, and have been done much more fully as we set ourselves the task of finding out what part of the nuclein acted especially on the leucocytes and the nature of that action.

One of us put himself on absolutely fixed medium diet and subjected himself as far as possible to the same conditions each day. The investigations were made in two series.

In the first after having remained on fixed diet for two days, 0.6 gm. powdered thymus tabloid was taken on the third day and 1 gm. on the fourth day.

In the second series, fixed diet was maintained for three days, 0.8 gm. thymus tabloid was taken on fourth day, 0.8 gm. on fifth day, and 1 gm. on sixth day. We were unable to try the action of larger

¹ Cp. Mayer. *Deutsche med. Wochenschrift*, 1896, S. 186-188.

doses as the amount given on the sixth day caused some general disturbance.

The methods which we employed were as follows:—

The total urine of twenty-four hours was carefully collected, mixed and examined in the following way. For the estimation of the *nitrogen* and *phosphorus* the Kjeldahl-Weibull method was employed, viz.—20 c.c. of urine were run from a burette into a Kjeldahl flask, then 10 c.c. H_2SO_4 , ten grammes K_2SO_4 and a few crystals of CuSO_4 were added and the mixture incinerated in the usual way. The clear green fluid left after complete incineration was then made up to 300 c.c. with distilled water, and of this, 75 c.c. were taken for nitrogen and the remainder for phosphorus.

The *nitrogen* was estimated by the ordinary method, the ammonia being distilled over into a measured amount of fifth-normal oxalic acid and titrated in the usual way.

The part taken for *phosphorus* was generally evaporated down to smaller bulk for convenience in filtering, ammonia was added till alkaline, then nitric acid till it again became acid—after cooling, ammonium-molybdate solution was added and the beaker allowed to remain in a warm place over night. The fluid was then filtered and the yellow precipitate washed with the filtrate on to the filter-paper, and then finally washed with dilute nitric acid. The filtrate was tested with ammonium-molybdate solution in order to see whether all the phosphate was precipitated. The yellow precipitate was then dissolved in dilute ammonia, some magnesia mixture added, and the mixture again allowed to remain over night. The resulting precipitate of ammonio-magnesium phosphate was then filtered, washed chlorine free, incinerated and weighed. From the amount of magnesium pyrophosphate so obtained, the total P_2O_5 was calculated.

The *uric acid* was estimated by the well-known Salkowski-Ludwig method in the earlier observations; but, after the publication of Salkowski's recent paper¹, we followed the modification there described. The ordinary Salkowski-Ludwig method does not require description here. The nitrogen of the uric acid crystals was estimated preferably to weighing them. In his later modification Salkowski advised that larger quantities of urine should be taken in order that the alloxur bases might also be estimated in the same specimen. About 700 c.c. of urine were taken, and the uric acid and alloxur bases precipitated with the magnesia and silver solutions. The uric acid was

¹ *Pflüger's Archiv*, LXIX. S. 268–306. 1898.

then set free, and the fluid containing all the alloxur bodies evaporated down to dryness. The residue was treated with warm dilute sulphuric acid which dissolved out the bases. The mixture was then allowed to stand over night, filtered, the crystals of uric acid washed, dried and the nitrogen estimated by Kjeldahl; the filtrate containing the alloxur bases was made ammoniacal, silver nitrate added, and the precipitate so obtained filtered, washed chlorine, silver, and sulphate free and incinerated in a porcelain capsule. The ash was then extracted with dilute nitric acid and the silver estimated by titration with NH_4CNS . From the amount of silver so obtained, the N. of the alloxur bases is calculated from the formula representing the sum of the Ag. combinations of these bases.

*Camerer's method*¹, which we employed in part of our observations for estimating the *nuclein bases*, corresponds to the above methods to some extent, the alloxur bodies being precipitated by magnesia and silver in the same way. The precipitate was filtered, washed ammonia free on a suction-pump filter and its nitrogen estimated (Kjeldahl). The figure so obtained was held to be the amount of alloxuric nitrogen—the difference between this and that of the uric acid representing the nitrogen of the alloxur bases. In his paper (*loc. cit.*) Salkowski criticises this method and with right, pointing out that the amount of urine used (equivalent to 100 c.c.) was far too small for accurate results, and that the precipitate contained more nitrogen than that due to the alloxur bodies, namely, some ammonia which united with the uric acid and which could not be removed unless by such an amount of washing as would dissolve the silver xanthin to a considerable extent. We have employed both methods in a very large number of cases and have found the Salkowski one to be the more reliable.

The total bases were estimated as follows:—Fifty c.c. of urine were taken and dilute HCl and phosphotungstic acid (1–10) added till no more precipitate was thrown down. After standing twenty-four hours, it was filtered and the precipitate washed with dilute sulphuric acid until the volume of the filtrate amounted to 300 c.c. The nitrogen in 30 c.c. of this fluid was then estimated (Kjeldahl). The difference between this and the total amount of nitrogen in 5 c.c. urine gave the nitrogen of the total bases of that amount of urine². We estimated

¹ *Zeit. f. Biol.* xxvi. S. 104, 1893; xxvii. S. 153, 1890; xxviii. S. 72, 1891; xxix. S. 231, 1892.

² For the nature of the bodies precipitated by phospho-tungstic acid see papers by v. Hirschler, *Zeit. f. physiol. Chemie*, xi. S. 25, 1887; Hofmeister, *Zeit. f. physiol. Chemie*, v. S. 67, 1881.

the total bases in order that we might see whether any other substances outside the alloxuric bodies were altered in amount after giving nucleic acid—it being possible that some other base might be excreted which was not precipitated by silver.

TABLE I. ANALYSIS OF URINE IN METABOLISM UNDER THYMUS.

<i>First Series.</i>						
Date	Quantity	Specific gravity	Total nitrogen as N grms. per diem	Total phosphorus as P_2O_5 grms. per diem	N. of uric acid grm. per diem	N. of alloxur bases grm. per diem (Cammer)
March 2	864	1027	15.144	3.020	.157	.09
3	920	1026	15.301	2.980	—	—
4	783	—	13.689	3.132	.146	.09
5	830	1027	14.362	3.130	.203	.05
Averages	(a) on fixed diet (b) on thymus		15.225 14.025	3.000 3.131	.157 .174	.09 .07
<i>Second Series.</i>						
Mar. 9	700	1028	12.961	2.656	.226	—
10	1090	—	12.757	2.557	.170	.1
11	947	1024	13.420	2.731	.216	.09
12	857	1027	11.950	2.514	.151	.11
13	914	1024	13.387	2.791	—	—
14	870	1018	12.143	3.034	.195	.07
15	980	1025	13.390	3.091*	.195	.09
16	865	—	11.443	1.733	—	—
17	782	—	10.756	1.987	.273	—
Averages	(a) on fixed diet (b) on thymus		12.455 12.493	2.459 2.780	.216 .173	.093 .090

* The rise on this day is evidently due to the action of the thymus given on the afternoon of the previous day.

We shall here describe the methods employed for the analysis of the fæces, although these were examined only in the nucleic acid and metaphosphoric acid investigations. The total amount was weighed,

and in a specimen from the mixed fæces the water, N. and P. estimated in the usual way. For the estimation of the alloxur bodies a large quantity (70 to 100 grammes) was boiled with 2% H_2SO_4 for 7 to 8 hours. The mixture was then made alkaline with hot baryta water, filtered, and then rendered barium free. The filtrate was now made ammoniacal, magnesia mixture added, and then precipitated with silver nitrate. From the precipitate the alloxur bases were estimated by Salkowski's recent method which we have just described. On no occasion did we find uric acid present in the fæces.

The points of special importance in this series of experiments are:

(1) There is no doubt that the P_2O_5 excretion is increased even when very small doses of thymus are given. Thus, in the first series, after giving 0.6 g. thymus tabloid the P_2O_5 excretion rose from 3.00 g. (average over two days on fixed diet) to 3.131 g., while only 0.013 g. P_2O_5 was present in the substance given. This shows that 0.127 g. P_2O_5 was excreted above that derivable from the phosphorus present in the tabloid even supposing that all was absorbed. The same holds of the phosphorus excretion during the second series, when thymus was again given (see Table I.).

(2) Relatively also the P_2O_5 is increased in proportion to the nitrogen. The ratio under normal conditions in the first series being 5.07 N : 1 P_2O_5 , while after thymus the ratio is 4.47 N : 1 P_2O_5 . In the second series the fixed diet ratios were 5.06 : 1, while the thymus ratios were 4.58 : 1 (Table II.).

(3) With the small amount of thymus taken there was practically no appreciable alteration in the excretion of the alloxuric bodies either absolutely or relatively to the total nitrogen or total P_2O_5 .

For the quantities of the different substances excreted see Table I.

TABLE II. METABOLISM UNDER THYMUS.

Table of Ratios.

	$\text{P}_2\text{O}_5 = 1 : \text{Total N}$	Alloxuric N = 1 : P_2O_5	Alloxuric N = 1 : Total N
<i>First Series.</i>			
(a) on fixed diet	1 : 5.07	1 : 12.1	1 : 61.3
(b) under thymus	1 : 4.47	1 : 12.6	1 : 57.8
<i>Second Series.</i>			
(a) on fixed diet before & after giving thymus	1 : 5.06	1 : 9.74	1 : 4.60
(b) under thymus	1 : 4.58	1 : 10.50	1 : 4.57

Metabolism of Nucleic Acid.

This was for us much the most important part of the work, and has been done more fully than in the case of the thymus. It was important to find out whether it was the phosphorus-holding acid present in the nuclein which acted as a disturber of the nitrogen and phosphorus metabolism.

The laboratory steward (R. B.) kindly agreed to go on fixed diet during the course of the investigation. The urine and fæces were examined for five days when fixed diet alone was taken. The leucocytes were numbered at a fixed hour each day shortly before the mid-day meal. Throughout the whole investigation the analyses of the urine comprised total nitrogen, total P_2O_5 , N. of uric acid, N. of alloxur bases, N. of total bases. The nitrogen, P_2O_5 , uric acid and alloxur bases of the fæces were also estimated. On the sixth day 0.5 g. nucleic acid (in weak alkaline solution) was given at 8 a.m., and on the following day 1 g. at 9 a.m. On the succeeding three days fixed diet alone was taken.

The points of special importance in this series are :

(1) That the P_2O_5 excretion in the urine is increased absolutely on giving even small doses of nucleic acid. In order to avoid any fallacy from daily variations in the amount of phosphorus excreted, it is better to take the average amount of P_2O_5 over the eight days on fixed diet and compare this with the average daily excretion when nucleic acid was taken. Thus, 4.038 g. P_2O_5 was the average excretion on fixed diet, 6.162 g. on nucleic acid. When one remembers that the average amount of P_2O_5 taken in in the form of nucleic acid was only 0.17 g. then if all the phosphorus of the acid were absorbed, there would still be an excess of 1.954 g. P_2O_5 derivable from phosphorus-holding bodies in the tissues. The examination of the fæces showed that all the phosphorus-holding part of the nucleic acid was absorbed, seeing that there was certainly no increase in the amount excreted by the bowel (Table III.).

(2) In proportion to the nitrogen the P_2O_5 is also relatively increased, the ratios of P_2O_5 to nitrogen being, for the eight days on fixed diet, 5.12 : 1, on nucleic acid, 3.70 : 1 (Table IV.).

(3) With regard to the alloxur bodies excreted in the urine one has to be careful in drawing conclusions as to the action of nucleic acid.

Although the alloxuric nitrogen in the urine appears to be slightly increased, the amount of the increase might be accounted

TABLE III. METABOLISM UNDER NUCLEIC ACID. (R. B.)

Urine										Feces						
Date	Quantity (c.c.)	Sp. gr.	Re- action	Total N	Total P ₂ O ₅	N of uric acid	N of alloxur bases	N of total bases	Quantity (dried) %	Water %	Total N	Total P ₂ O ₅	N as alloxur bases	Leucocytes	Remarks	
May 11	1683	1.025	acid	19.584	4.247	0.240	0.007	—	25.90	73.81	1.820	0.640	—	—	Fixed diet	
12	2356	1.025	"	22.072	4.138	0.278	0.006	2.992	37.40	71.83	2.682	1.203	0.025	—	"	
13	1695	1.025	"	23.134	4.387	0.284	0.009	2.489	60.50	70.19	5.997	3.086	0.018	8750	"	
14	2157	1.022	"	20.783	3.696	0.298	0.010	1.070	14.01	70.82	1.039	0.518	0.025	6500	"	
15	2125	1.024	neut.	26.413	4.648	0.205	0.026	3.407	33.61	69.11	2.519	1.175	—	9000	"	
16	2087	1.022	acid	23.341	6.324	0.257	0.023	2.273	29.78	74.33	2.328	1.146	0.039	10,500	5 gr. nucleic acid	
17	2848	1.018	"	22.344	6.000	0.315	0.025	3.598	28.08	71.35	2.035	0.997	0.039	11,250	1 gr. " "	
18	1461	1.027	"	19.875	4.136	0.162	0.016	2.580	41.34	71.69	3.238	1.533	0.018	9000	Fixed diet	
19	2222	1.024	"	25.632	5.397	0.307	0.032	1.206	27.43	69.19	2.094	1.047	0.021	8000	"	
20	1685	1.027	"	17.203	3.402	0.232	0.024	—	40.22	68.58	2.894	1.391	—	9000	"	
Averages (a) on fixed diet				21.648	4.038	0.252	0.017	2.191								8250
(b) on nucleic acid				22.842	6.162	0.286	0.024	2.985								10,850

* We consider that these figures are abnormally low as more prolonged examination of this urine excreted under fixed diet gave an average of about .02 gm. N in form of alloxur bases.

for by that part of the bases which has been absorbed from the nucleic acid.

TABLE IV. METABOLISM UNDER NUCLEIC ACID.

Table of Ratios.

	$P_2O_5 = 1 : \text{Total N}$	$\text{Alloxuric N} = 1 : P_2O_5$	$\text{Alloxuric N} : \text{Total N}$
(a) on fixed diet	1 : 5.25	1 : 12.85	1 : 80.1
(b) when nucleic acid given	1 : 3.70	1 : 19.8	1 : 73.7

This does not prove that the alloxur bodies in the urine on the "nucleic acid" days have not been derived from other sources than the acid given, viz., leucocytic breaking down. What in all probability occurs is, that the absorbed products of nucleic acid cause hyperleucocytosis with a consequent increased leucolysis, the result being, that there is an increase in formation of alloxur bases, but these undergo further changes and do not arrive in the urine entirely as such.

We base our view of the increased leucolysis after nucleic acid on the rise in the P_2O_5 excretion, the nature of which has just been discussed.

(4) Of the total alloxuric nitrogen, it seems to be the uric acid which is most markedly increased after nucleic acid. The increase, however, is so slight, that one has to be exceedingly cautious in drawing conclusions (vide note at foot of Table III.).

(5) The total bases precipitable by phospho-tungstic acid were on an average slightly increased after nucleic acid, but here again the daily variations were so great that one can hardly lay much stress on the excretion during any two isolated days.

(6) There is absolutely no doubt that the acid produces a hyperleucocytosis within four hours after its ingestion, and an accompanying or subsequent leucolysis.

Metabolism of metaphosphoric acid.

Seeing that nucleic acid acts along the same lines as the nucleins or nuclein-holding tissues, the next question was, to what constituent of the acid is the action due?

The next body that one would naturally examine is thymic acid. Quite recently Neumann¹ referred shortly to a leucocytosis produced

¹ Abstract from *Deutsche med. Wochenschrift*, S. 100, May 26, 1898. Neumann, *Physiol. Gesell. in Berlin. Sitzung*, 29 April, 1898.

by thymic acid; but we have only seen a very short abstract of his paper by Loewy and so are unfortunately unable to speak further about it. We intend, however, to investigate the action of this acid immediately and hope to be able later to give the results of our work. Neumann speaks of a nucleo-thymic acid as distinct from thymic acid. The reason for this we do not understand, as what was previously called by Kossel paranucleic acid (derived from nucleic acid by splitting off the bases) is now regarded by him as identical with thymic acid. A point of importance, which Neumann refers to, is that hypoleucocytosis in this case does not precede the hyperleucocytosis. The importance of knowing how this acid acts is apparent, seeing that it is free from alloxur bases.

The next constituent of the nuclein which we examined is a hypothetical one, viz., monometaphosphoric acid. As such, it is probably not present in the radicle of nucleic acid, but as a polymer of it, perhaps trimetaphosphoric acid. It is important to know its action, from, among other reasons, the fact that Liebermann regards the nucleins as combinations between metaphosphoric acid and albumin. The methods employed in the investigation were exactly similar to those described for nucleic acid, so that now we can go on directly to a discussion of the results as shown in Table V.

I. When we take the average P_2O_5 excretion over four days on fixed diet, and compare it with that occurring on the two days when metaphosphoric acid was given, one sees at once that there is an absolute increase in the latter case. Here, however, the increase is of a different kind from that met with after giving nucleic acid. Thus, the average excretion under fixed diet was 4.208 g., while that on HPO_3 was 5.065 g., that is to say, an increase of 0.757 g., P_2O_5 . Now the amount of P_2O_5 taken in the form of HPO_3 amounted to, on an average over the two days, 1.325 g. The increase in the P_2O_5 excreted in the urine in the case of HPO_3 is less than that taken in by the mouth, while in the case of nucleic acid exactly the opposite occurs. The deficit in the former case amounted to 0.568 g. The question comes to be, is this balanced by the increased P_2O_5 in the fæces? In this instance as an examination of Table V. shows, the phosphorus in the fæces is not increased until the following day in each case, a distinct rise occurring also on the third day.

II. With regard to the relative amounts of P_2O_5 and nitrogen, the ratio on fixed diet was 5.06 : 1, that on HPO_3 , on the other hand being 4.95 : 1, the increase, as one sees, being an exceedingly small

TABLE V. METABOLISM OF METAPHOSPHORIC ACID (R. B.)

Urine										Fæces					
Date	Quantity	Sp. gr.	Re- action	Total N	Total P ₂ O ₅	N of uric acid	N of alloxur bases	N of total bases	Leuco- cytes	Quantity (water free)	Water %	Total N	Total P ₂ O ₅	N as alloxur bases	Remarks
June 1	1925	1.018	acid	21.560	4.669	0.239	0.031	2.372	8400	27.571	68.31	2.001	2.054	0.030	Fixed diet
2	1845	1.020	"	19.940	4.376	0.262	—	1.963	8000	31.490	72.86	2.223	2.429	0.027	"
3	2182	1.020	"	23.949	5.097	0.220	0.038	1.711	8000	21.880	72.66	1.577	1.731	0.018	.5 gm. HPO ₃
4	2001	1.020	"	26.211	5.033	0.272	0.022	5.169	8500	29.930	71.23	2.029	2.563	0.035	1 gm. HPO ₃
5	1443	1.025	"	19.636	3.499	0.135	0.032	3.119	8000	28.010	70.52	2.360	2.517	0.024	Fixed diet
6	2072	1.020	"	23.589	4.291	0.263	—	2.704	6750	35.720	72.53	2.696	3.510	0.028	"
7									8250						
Averages.															
(a) on fixed diet				21.181	4.208	0.224	0.031	2.539	7860						
(b) " + HPO ₃				25.080	5.065	0.246	0.030	3.440	8250						

one, not at all comparable to that noticed in the nucleic acid experiments. (Table VI.)

TABLE VI. METABOLISM UNDER METAPHOSPHORIC ACID.

Table of Ratios.

	$P_2O_5=1$: Total N	Alloxuric N=1 : P_2O_5	Alloxuric N=1 : Total N
(a) on fixed diet	1 : 5.06	1 : 19.1	1 : 83.06
(b) on metaphosphoric acid	1 : 4.95	1 : 18.4	1 : 90.87

III. The alloxuric excretion (bases + uric acid) is practically unaffected when one looks at the absolute quantities, while relatively in proportion to the total nitrogen, it is diminished as one would expect in the case of such a mineral acid where the proteid metabolism is increased.

IV. The leucocytes showed no rise in numbers.

Metabolism of the nucleins under pathological conditions.

We now go on to a study of the metabolism of the nucleins under pathological conditions, and here, although our investigations have been very extended, we wish to exercise caution in drawing conclusions.

The interesting connection between pathological conditions showing hyperleucocytoses and the excretion of bodies in the urine derivable from the decomposition of nucleins has been emphasised recently by a large number of writers. We think, however, that the connections between leucocytosis and the excretion of the alloxur bodies are not capable of such an easy explanation as many have put forward.

Leucocytoses occurring in the most diverse conditions (*e.g.* in pneumonia, Hodgkin's disease, carcinoma of different organs, experimental leucocytoses from the action of different substances injected into blood stream or given by the mouth, not to speak of innumerable cases of leucocythæmia) have been grouped together, and a close relationship shown in all cases between the rise in the number of the leucocytes and the rise in the alloxuric excretion. Such a view can only be held when the writers believe that the absolute amount of nuclein decomposition increases in the same proportion as the rise in the number of the leucocytes. In other words, leucocytosis and leucolysis go hand in hand; but as we shall show immediately there is in all probability an increase in leucocytes due to diminished breaking down. In such a case one could not expect the alloxuric nitrogen or,

more important, the P_2O_5 to be increased. Even if the former were increased with the rise in leucocytes, one could not simply draw the conclusion from this that the nuclear decomposition was raised.

Although the amount of work on this subject during the last few years has been immense, still our knowledge of the nature of the different forms of leucocytoses is still very far from complete.

Either the methods of analysis have been faulty as, *e.g.*, the estimation of alloxur bases by the Krüger-Wulff method or the amounts of urine taken for the analysis of the latter have been far too small.

Dunin and St Nowaczek¹ have estimated the uric acid before and after the crisis in croupous pneumonia and have found it increased shortly before, most markedly, however, just after the crisis, returning to the normal about the seventh or eighth day. He used Haycraft's method for uric acid estimation, which unfortunately is not an entirely reliable one. The experiments do not teach us much, as neither the P_2O_5 nor the alloxur base excretion is given.

Kühnau and Weiss² examined a large number of cases presenting different forms of leucocytosis, and gave as the result of their investigations that an intimate relationship existed between leucocytosis and the alloxuric excretion, viz., an increase in the former resulting in a corresponding rise in the latter. Here no attention has been paid to the P_2O_5 excretion, nor to the ratios existing between total N, alloxuric N, and P_2O_5 .

The first pathological condition which we shall take up is that of leucocythæmia. Here there is not only a marked increase in the number of the white blood corpuscles, but an alteration from the normal in the proportion of polynuclear to mononuclear forms. Now the question was, is this qualitative alteration in the leucocytes a disturbance in their life history? As there is no certain sign either in the blood or blood-forming organs of an increased production of white blood corpuscles in leucocythæmia, one is almost led to believe that the fundamental alteration is a hindrance in the transformation of mononucleated into polynucleated cells. If this arrest actually occur, then one would expect that an examination of the urine would show, instead of a rise in P_2O_5 corresponding to a hyperleucocytosis, a diminution of the same. The reason for the passage of the myelocytes (Ehrlich) from the bone-marrow into the blood, whether it be due to

¹ *Zeit. f. klin. Med.* xxxii. S. 1, 1897.

² Kühnau. *Zeit. f. klin. Med.* xxviii. S. 534; Kühnau and Weiss, *ibid.* xxxii. S. 482, 1897.

active movements on the part of those cells, or mechanical transmission of them by means of the blood stream, it is impossible at present to decide. Ehrlich holds that the former is the more probable¹.

From what we shall immediately show, there is no doubt that the life-history of the leucocytes in leucocythæmia, looked at from the chemical standpoint, is absolutely different from that in the form of "active" (nucleic acid) leucocytoses which we have examined.

The points to be specially noted in this case of leucocythæmia are those which have been referred to in the case of the nuclein investigations. At the outset we wish to avoid exaggerating the importance of diurnal variations in the alloxuric excretion compared to increase or decrease in the number of leucocytes. Rather would we try to find out from the average of a very large number of analyses the nature of the fundamental changes in the metabolism of the nucleins in this condition.

(1) The average amount of P_2O_5 excreted in the urine in this case (over the analyses of twenty days) was 0·915 g., while under normal conditions in the case of R. B. (vide Table III) the average excretion was 4·038 g. When one remembers that the average number of leucocytes under normal conditions in the case referred to above (R. B.) was 8,000, in the leucocythæmic case on the other hand 330,000, then, if the amount of P_2O_5 be any gauge of the nuclear breaking down, one is compelled to admit that in the latter case the leucolysis is diminished (Table VII).

(2) As in the previous investigations it is important to find out whether the nitrogen and phosphorus metabolism is disturbed, as shown by alterations in their relative proportions.

The average ratio in this case was 9·17 N : 1 P_2O_5 , while in the normal it was 5·25 : 1. That is to say, not only is the P_2O_5 absolutely, but also relatively diminished. This is another proof of the diminution of the leucolysis (Table VIII).

TABLE VIII. TABLE OF AVERAGE RATIOS.

Conditions	$P_2O_5=1$ to Total N	Alloxuric N = 1 : P_2O_5	Alloxuric N : Total N	Leucocytes
Normal (R. B.)	1 : 5·25	1 : 12·85	1 : 80·17	8000
Thymus	1 : 4·58	1 : 12·60	1 : 57·80	—
Nucleic acid	1 : 3·70	1 : 19·80	1 : 73·70	10850
Metaphosphoric acid	1 : 4·95	1 : 18·40	1 : 90·80	7860
Leucocythæmia	1 : 13·28	1 : 1·905	1 : 25·3	330000
Plumbism	1 : 4·82	1 : 11·60	1 : 56·2	11430

¹ Ehrlich u. Lazarus. *Die Anæmie. Erste Abteil. Nothnagel's Pathologie u. Therapie*, 1898.

TABLE VII. LEUCOCYTHÆMIA (SPLENO-MEDULLARY). (TRAVERS.)

Date	Total urine of 24 hours in c.c.	Specific gravity	Total nitrogen (as N) grms per diem	Total phosphorus (as P_2O_5) grms per diem	Total nitrogen of uric acid grms per diem	Total nitrogen of alloxuric bodies (Camerer)	Total nitrogen of bases	Leucocytes
Oct. 28	2200	1.015	16.016	2.25	0.294	—	—	—
29	860	1.017	6.935	0.97	0.208	0.307	0.099	400,000
31	910	1.018	—	0.83	0.142	0.323	0.181	—
Nov. 1	1730	1.014	—	1.11	—	—	—	480,000
2	1500	1.012	7.980	0.60	0.252	0.495	0.243	400,000
3	1480	1.013	8.619	0.96	0.297	0.442	0.151	408,000
4	1100	—	5.718	1.04	0.247	0.341	0.093	304,000
5	912	1.018	8.069	0.76	—	0.234	—	340,000
6	560	1.016	4.421	0.17	0.128	0.192	0.064	300,000
7	805	1.014	7.393	0.48	0.092	0.199	0.107	—
9	1140	1.018	10.916	0.57	0.276	0.354	0.078	—
10	1090	1.015	9.919	1.03	0.133	0.399	0.266	296,000
11	1090	1.016	9.804	1.08	0.231	0.288	0.056	208,000
12	773	1.018	6.410	0.945	0.172	—	—	298,000
14	872	1.017	5.490	0.590	0.222	0.366	0.144	—
15	996	1.017	10.597	1.400	0.167	0.262	0.094	415,000
16	842	1.017	9.900	0.693	0.208	0.338	0.130	305,500
18	895	1.017	10.475	0.762	0.177	0.320	0.143	217,000
19	1142	1.017	11.831	1.362	0.185	0.434	0.249	254,000
21	1111	1.016	9.083	0.707	0.209	0.351	0.142	—
Averages			8.397	0.915	0.202	0.332	0.140	330,000

¹ On these days the P_2O_5 was estimated by the uranium method, and the third decimal figure has been omitted as unreliable.

(3) The average total alloxuric excretion amounted to 0.332 g. N., while under normal conditions (R. B.) the excretion was 0.270 g. There seems to be but little doubt that, in this case, where the total nitrogenous excretion was exceedingly low, the alloxuric excretion was certainly relatively increased.

We can lay no stress on the excretion of the nuclein bases *per se*, as they were estimated by Camerer's method. In the majority of cases of leucocythæmia published by others there has been a more marked alloxuric increase.

(4) The ratio between the P_2O_5 and the alloxuric nitrogen is, as can be seen from Table IX, absolutely different from that existing normally. This alteration may be due to two conditions, a diminution in the phosphorus, along with an increase in the alloxuric nitrogen.

For other points in connection with the absolute amounts of the different nitrogenous constituents in the urine (see Table VII).

Plumbism.

We next take up another form of pathological leucocytosis where the white blood corpuscles are increased in number, but show no distinct alteration in the proportion of mono- to poly-nucleated forms.

When we compare it with the other conditions along the same lines we find:—(Table IX).

(1) The P_2O_5 shows no distinct absolute increase, nor is the ratio between P_2O_5 and N. altered from the normal.

The variations in the amounts of the alloxur bases are undoubtedly due to the fact that Camerer's method was here employed. The uric acid excretion is about the normal.

(2) There is one important point as forming a marked contrast to the leucocythæmic case, namely, that the alloxuric and P_2O_5 excretions correspond to the normal.

(3) The leucocytes did not alter greatly in number during the course of the investigations, and never at any time was the leucocytosis a marked one.

This may be one of the reasons why the nitrogen and phosphorus metabolism shows no marked aberrations from the normal, another complicating factor being that the patient suffered from chronic Bright's disease. During the course of the investigations the patient was passing fairly large quantities of lead in his urine.

TABLE IX. CASE OF PLUMBISM. (HOLLINSHEAD.)

Date	Quantity	Specific* gravity	Total nitrogen	Total P ₂ O ₅	Nitrogen of uric acid	Nitrogen of alloxur bases (Camerer)	Alloxuric nitrogen	Leuco- cytes
Dec. 1	1143	1018	8.540	1.738	0.083	0.032	0.126	10,625
2	1817	1018	12.260	2.956	0.151	0.089	0.241	11,250
3	1780	1016	13.506	2.564	0.159	0.061	0.221	10,937
5	1800	1015	11.94	2.358	0.150	0.063	0.214	7,500
6	1734	1015	11.79	2.358	0.126	0.084	0.211	9,375
7	1781	1016	12.06	2.010	0.149	0.058	0.208	14,375
8	1953	1014	13.12	3.320	0.147	0.063	0.210	11,560
9	1961	1015	14.60	2.702	0.149	0.092	0.241	7,500
10	1460	1015	11.17	3.206	0.130	0.051	0.182	7,812
12	1935	1017	14.03	4.419	0.150	0.103	0.254	10,625
13	1964	1015	14.29	3.606	0.150	0.093	0.244	13,749
14	2091	1014	16.448	3.600	0.111	0.164	0.275	13,437
16	1925	1014	19.080	4.612	0.125	0.006	0.131	14,062
19	1825	1015	11.293	2.074	—	—	—	11,600
21	1120	1015	9.909	1.763	0.035	0.14	0.175	10,000
22	1210	1015	9.079	1.637	0.171	0.14	—	—
23	2124	1015	9.277	3.338	0.205	0.324	0.529?	—
26	1710	1012	11.108	1.756	0.144	0.315	0.459?	11,300
27	1501	1015	16.567	—	—	—	0.322?	11,000
28	1750	1013	12.838	2.862	—	—	—	11,875
29	1380	1012	9.032	1.841	—	—	—	10,000
30	1870	1015	13.508	.716	—	—	—	12,746
Jan. 2	1620	1012	15.694	1.511	—	—	—	12,812
3	1880	1012	12.29	2.734	—	—	—	10,000
4	2060	1011	14.5	2.633	—	—	—	12,187
5	1850	1018	13.3	2.358	—	—	—	12,812
6	1393	1016	14.3	1.999	0.105	0.037	0.142	10,937
7	1300	1014	9.36	2.121	0.105	0.035	0.140	12,187
9	1194	1016	8.79	2.702	6.097	0.047	0.144	14,000
11	1652	1017	11.7	3.068	0.141	0.127	0.268	10,000
12	2115	1016	18.59	2.962	0.155	0.032	0.187	10,312
13	1797	1014	12.59	2.463	0.130	0.068	0.198	16,249
14	1692	1015	12.54	2.793	0.156	0.058	0.224	10,937
16	1640	1015	12.07	2.656	0.115	0.097	0.212	10,937
17	1583	1017	10.95	2.610	0.085	0.091	0.176	14,377
18	1678	1017	13.64	3.343	—	—	0.218	11,875
19	1514	1018	11.5	1.969	—	—	0.183	11,562
20	2073	1016	14.24	3.664	0.137	0.092	0.229	11,874
21	1310	1015	10.0	2.29	0.109	0.073	0.182	12,810
Averages			12.590	2.610	0.131	0.093	0.224	11,430

* The reaction was acid throughout.

CONCLUSIONS.

(1) The digestion products of nuclein-holding tissues, nucleins and nucleic acid cause, on being absorbed, a temporary leucocytosis which is accompanied by a rise in the P₂O₅ excretion above that derivable from the absorbed phosphorus. These alterations are especially well marked after giving nucleic acid.

(2) The alloxuric bodies are excreted in excess, after nucleic acid

has been given, and in all probability also after large doses of nuclear-holding tissues or nucleins, although in our experiments, owing to the small amount of thymus taken, there was no distinct increase.

(3) The uric acid excretion after nucleic acid was only slightly, if at all, increased. We were exceedingly anxious to give larger doses of nucleic acid but were unable to do so because of certain rather disagreeable symptoms (severe muscular tremors) which arose after the larger quantity had been given.

(4) Metaphosphoric acid has neither the action upon the leucocytes nor on the P_2O_5 excretion that was noticed in the case of nucleins and nucleic acid.

(5) The excretion of alloxur bodies is not altered in amount after metaphosphoric acid has been given.

Whether the action on the leucocytes is lost with the disappearance of the alloxur bases from the nucleic acid, or whether thymic acid is still able to act in the same way we are not yet in a position to say, although Neumann's experiments seem to prove that this latter acid has a leucocytotic action.

(6) The action upon the leucocytes is in all probability the primary factor leading to the alteration in the P_2O_5 excretion, that is to say, subsequently to, or accompanying, the hyper-leucocytosis there is a leucolysis.

(7) The action of nucleins, etc., on the excretion of the total bases is not a constant one.

(8) In the case of leucocythæmia examined the condition seemed to consist in a hindrance to the leucocytic breaking down as evidenced by an absolute diminution of P_2O_5 , and a marked relative decrease of the same in proportion to the total nitrogen.

(9) The alloxuric nitrogen as a whole was, relatively to the total N, increased in the leucocythæmia case, both the bases and uric acid apparently participating in this. It serves no good purpose to propound a theory to explain this diminution in P_2O_5 , compared to the relative increase in alloxuric nitrogen. We may merely say that undoubtedly in leucocythæmia the developmental history of leucocytes is different from that existing under normal conditions.

(10) In plumbism the conditions are comparable to those existing normally, the leucocytes being merely altered in number and not with regard to the relative proportions of the different varieties.

(11) In all cases where the fæces were examined uric acid was found to be absent, and we can hardly understand how its appearance

can have the significance which Weintraud (*loc. cit.*) believes it to possess.

Although Dr Low kindly enumerated separately the mononucleated and polynucleated leucocytes in the case of plumbism we abstained from giving the results here as they have no bearing on the work in the present paper.

We have to thank Professor Rutherford, in the first place, for his kindness in furthering our work at all times, also Professor Greenfield for granting us permission to examine the urine in the case of plumbism, and Dr James for allowing us to investigate the case of leucocythæmia.

We are also indebted to Dr G. C. Low and Mr J. J. Galbraith for their kindness in estimating the leucocytes so carefully.

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