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INVESTIGATIONS ON ARTIFICIAL RESTRICTION OF PROTEIN METABOLISM IN FEBRILE SHEEP

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(with 3 curves)

Arch f. exp. Path u Pharm., 47, 1902, pp 19-47.

It has been demonstrated beyond doubt that increased protein catabolism in a febrile organism can be limited by ample supply of food. But so far it has only been possible to abolish it completely in rare cases.

It is assumed that inanition which accompanies fever is responsible for the exchangeable fraction of protein loss while the other fraction is due to the fever-producing toxins. Recently attempts have been made to restrict or abolish the protein loss due to the latter by supplying large quantities of carbohydrates. If this should be possible in the same manner and to the same extent as it is in the fasting healthy individual[1] then certain concepts can be formed on the causes of this part of abnormal nitrogen excretion because it too would be replaceable just like the fraction of protein catabolized during fasting. It must be emphasized, however, that equal quantities of carbohydrates given during fever then ought to reduce the protein metabolism at the same rate as is the case in the healthy individual[2]. This requirement, clearly formulated by Muller, has not yet been met because the findings

1) Compare E. Voit, Zschr. f. Biol., New Series, 14, Vol 334, pp 103 and 15 (1896).
2) Fr. Muller, Allg. Pathol. d. Ernaehrung in v. Leyden's Handbook I., p 179.

by Hirschfeld[1]) in experiments in which he obtained a nitrogen equilibrium or even protein repletion in febrile tubercular patients, have been reported with such scarcity of detail that -- in our opinion -- they cannot be utilized.

May[2]) succeeded in reducing protein metabolism by 15 and 47% respectively in his experiments on fasting rabbits afflicted with red murrain fever, by incorporating grape sugar into the stomach. However, extremely large quantities of sugar were administered. Rabbit D was given 97% of the entire energy exchange in the form of sugar, and rabbit C 69%. In each case sugar was fed on the last day of the experiment. Animal D had only a slightly raised temperature on that day, and in animal C heat formation was only 74% of that observed on the preceding day. To say the least, conditions in these experiments were rather complicated. It was highly desirable to continue such experiments for several days, the more so since von Noorden had already demonstrated that in short term experiments protein breakdown may be masked.

The problem of increased protein breakdown during fever is of considerable theoretical interest. We, therefore, used the opportunity for a renewed investigation when we learned from Councilor von Behring that he had produced high fever in cattle with a nasal mucus toxin in his possession, without simultaneously reducing the animals' appetite.

The fact that febrile animals or humans almost always lose their appetite presents an obstacle to all experiments of the type discussed above. If this complication can be excluded, experiments of a longer duration with ample feeding can be conducted. Councilor von Behring was kind to let us have large quantities of this toxin. We are greatly indebted to him for this kindness.

The following data on the properties of the toxin were obtained by us: dried, pure cultures of mucus bacilli were finely pulverized so that the bacterial bodies were destroyed. This powder was then extracted with water -- the solution used for injection contained approx. 50% of the dry bacterial substance. The solution was preserved with toluene. Unfortunately circumstances did not permit us to conduct our experiments on cattle. But the mucus toxin also generates high fever in dogs and sheep. The dog, however, completely lost his appetite while conditions in the sheep shaped up more favorably.

1) Hirschfeld, Berl. klin. Wschr. (1890) No 2.
2) Zschr. f. Biol., Vol 30 (1893).

Furthermore the sheep proved tc be very suitable for metabolism experiments along the lines which have been worked out in hundreds of experiments in Agricultural Experimental Stations. Councilor Kellner-Möckern and Prof. Dietrich-Marburg kindly put at our disposal their rich experience in the area of metabolism and feeding experiments, as well as in the processing of the fodder. Their kind assistance at all times is warmly appreciated.

Details of feeding are given in the chapter on Methods.

The investigations on metabolic processes after the injections, were conducted in such a manner that we first examined the effect of the fever on nitrogen metabolism in a sheep which was kept on maintenance rations and exhibited an approximate nitrogen balance. We next conducted an analogous experiment in a sheep in the state of considerable nitrogen gain, and, finally, approximating May's experimental arrangement, fever was induced in the animal after a 10-day fasting period and it was simultaneously fed with fodder containing ample amounts of nitrogen and carbohydrates.

Experiment No 1.

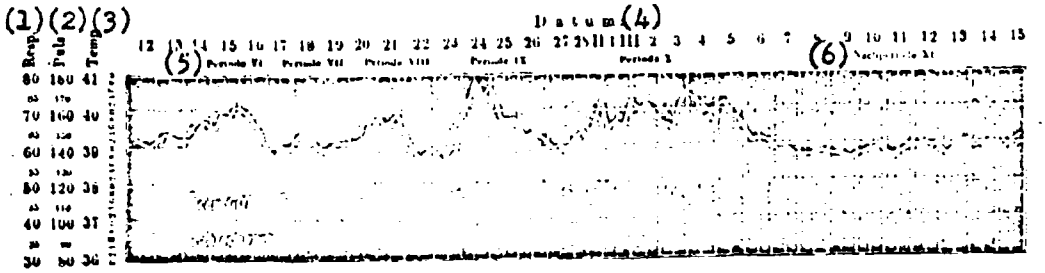
During the pre-experimental period the animal was given the following food daily: 1500 cc water, 400 g hay of medium quality, and 400 g rye bran, corresponding to 16.36 g N. Of this (subtracting residues) 9.7 g N were absorbed daily (-64% N of the fodder) and, calculating the caloric value of the absorbed nutritional substances, 1,758 calories[1]). This equals 49 cal/kg animal weight. Weight and N balance show that the animal was in a state of nitrogen and energy exchange equilibrium during the period 27 Jan - 13 Feb. Rectal temperatures fluctuated between 39.0-39.3°C. During the principal experimental period (14 Feb-5 March 1900) the animal's fever was as illustrated in Curve 1.

The animal now consumed 4/5 of the fodder offered, ingesting 11.76 g N daily of which 7.46 g N were absorbed (-63.2% of the nitrogen content of the fodder). 8.56 g N were excreted, i.e., an average of 1.1 g N -- approx 7 g protein loss daily. An amount of food having a value of 1,359 calories was absorbed, or 39 calories daily (calculated over the entire period). The

1) Compare Tract on Feeding by Wolff, 1899, Berlin. Tables pp 233-245. - It must be admitted that this calculation gives only approximate values. It is, however, not without value since possible errors are the same throughout all experiments and therefore relative figures are correct.

rate of protein turnover was that customary during fever. However, since the food consumption of the animal decreased during the fever period, and since in the pre-experimental period more protein and 10 calories more per kilo were available, this decrease in food uptake might at first be held responsible for the comparative increase in N excretion. And indeed such must be the case. But when we observe various periods within the entire span of time through which the fever lasted, we note that even after the sheep had become accustomed to the decreased food supply and especially to the reduced intake of protein, the amount of nitrogen excreted in the urine during high temperature nevertheless equals that excreted in the period preceding the fever. During the post-febrile period (6-23 March) the animal's daily nitrogen uptake was 7.7 g, it absorbed 66% of the protein offered. The absorbed portion of food contained 1,312 calories or 38 calories/kilo of body weight daily. The amount of food was thus almost the same as that during the preceding febrile period. Nevertheless, the animal put on 1.4 g nitrogen or 8.7 g protein daily. If the only influence exerted on the sheep during the period 14 Feb-5 March had been that of inanition, nitrogen loss should have occurred also -- at the same level of food supply -- during the period of 6-23 March. The fact that -- as we have stated -- nitrogen was retained during this period, proves that the febrile condition was primarily responsible for the nitrogen loss.

Curve 1.



1 -- Respiration; 2 -- Pulse; 3 -- Temperature; 4 -- Date; 5 -- Period; 6 -- Post-exp. period.

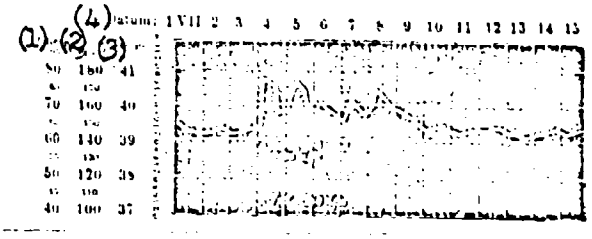
Experiment No 2.

We next attempted to produce fever in a sheep in a state of nitrogen gain in order to find out whether this state would continue during fever. Our task was to supply the animal with a great deal of protein, and in addition, during the fever period, the greatest possible quantities of carbohydrates. To

this end the animal was given daily 400 g hay, 500 g oats, 200 g cotton-seed flour, containing 31.1 g N and 2,867 calories, i.e., 73 calories/kilo of body weight (the digestibility of this feed has been taken into account). During the fever 50 g starch and 100 g lactose (the latter dissolved in the drinking water) were added. The lactose with the water was devoured greedily, but undeterminable amounts of starch mixed with other fodder residues were left. Disregarding the amount of starch taken up, 2,780 calories or 69 cal/kilo of body weight were absorbed daily. In reality, therefore, the animal consumed somewhat more.

Curve 2 below shows the temperature fluctuations.

Curve 2.



1 -- Respiration; 2 -- Pulse; 3 -- Temperature; 4 -- Date.

During the post-febrile period the animal was given the same feed as during the period preceding the fever. But during the last three days scraps of the offered food were left, so that the caloric supply has been calculated as 2,647 cal or 65 cal/kilo daily on the average.

75% of the protein given was utilized during the pre-febrile period, 70% during the main period, and 75% during the post-febrile period. These high figures are the result of our use of cottonseed flour for feeding, whose high degree of utilization has long been known to Agricultural Experimental Stations. The reduced absorption during the fever may be connected with the decreased utilization of raw proteins caused by increased carbohydrate supplies. According to experiments of E. Schulze and Marker this depression may affect up to 40% of utilizable protein.

During the pre-febrile period the animal gained an average of 7.0 g nitrogen daily, during the fever period 4.7 g, and in the post-febrile period 7.8 g. This shows, as has been known, that in the adult sheep considerable amounts of nitrogen-containing substance are retained by the organism, provided that uptake of food is sufficient.

Nitrogen excretion during the post-febrile period is relatively increased. This is an important factor in evaluating nitrogen retention during fever. It permits us to conclude beyond doubt that this retention in actual fact represents a gain and is not merely due to a deficiency in the excretion of nitrogenous end products. If it were otherwise the nitrogen previously retained would be excreted during the post-febrile period.

Undoubtedly then protein is retained even during the fever period. Unfortunately it is difficult to compare this with the preceding method. During the latter 145 g protein were absorbed, while only 105 g were absorbed during the febrile condition -- due to the animal's loss of appetite.

Turning our attention now to the caloric supply during the two periods, we observe that during the pre-febrile period where 23.4 g nitrogen were resorbed, 2,867 calories or 73 cal/kilo were taken up, while during the febrile period where 17.0 g N were absorbed, 2,780 calories[1], i.e., 69 cal/kilo were utilized. Thus the caloric differences of the food are very insignificant.

The considerable decrease in nitrogen assimilation may be due to decreased uptake of protein in spite of an almost undiminished uptake of calories. But when we compare the period 15-21 June (11.8 g of N absorbed daily and 2,270 cal, with a gain of 5.6 g nitrogen) with the fever period, during which nitrogen- and caloric-supply was considerably larger, while the gain was 1 g less per day, the considerable relative increase in nitrogen excretion during the fever becomes obvious.

Did the ample carbohydrate supply have no limiting effect on protein metabolism here? A comparison between the febrile period and the last three days of the 'after-period' elucidates this problem. During the latter period 0.6 g nitrogen more and 350 calories less were absorbed than during the former. This means that at approx the same nitrogen input the animal took up 117 calories less. The gain is 3.1 g daily, i.e., 1.6 g less than during the fever. In view of the higher caloric value of the food the febrile animal, despite its condition, thus had a 30% lower nitrogen turnover than it had during the 'after-period'. This is at least as much as the highest values which have been found in humans and animals for the so-called protein protection by carbohydrates. And this even though during the

1) In this calculation the calories resulting from the absorbed starch have been neglected. Had they been considered the figures would even be closer.

period used for comparison the animal was convalescing, i.e., it was in a favorable state for repletion.

Therefore, while during our main experimental period less protein was doubtless degraded as a result of the carbohydrate supply than would have been degraded without it, it is on the other hand apparent that more was used than would have been the case but for the febrile condition. It is possible to keep a febrile organism in a condition of 'gain' without, however, preventing protein breakdown -- in this case the animal uses up more protein than a healthy animal under otherwise similar conditions.

Experiment No 3.

The aim was to observe protein metabolism in a well-fed animal whose appetite was increased by a preceding period of fasting, which effect also made its cells suitable for protein repletion.

The animal was first kept in a condition of undernourishment for a while (5.5 g nitrogen were resorted daily, 6.8 g were excreted, i.e., 1.3 g body proteins were lost each day). It was then made to fast for 10 days. The temperature during this span of time was lower and less constant than normally. It fluctuated between 38.1° and 38.9°. During the last few days increasing weakness could be noticed.

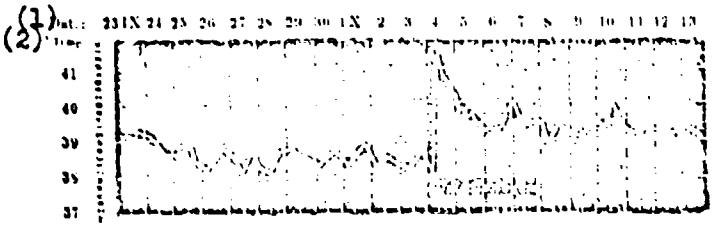
The behavior of nitrogen excretion in the urine was remarkable in that the values for the first days of the fasting period are considerably higher than those for the preceding days. Only on the fifth day the sheep's nitrogen excretion in the urine equaled that of the preceding period. Amounts of nitrogen then decrease further to 5.3 g. A total of 9.7 g nitrogen was excreted with the feces, i.e., on the average 1.0 g daily. During the fasting period the animal lost 416 g protein.

The fever period which followed lasted seven days. The sheep was fed immediately prior to injection of the mucus toxin. On the first day it consumed 400 g oats, 150 g cottonseed flour, and 75 g lactose, leaving no trace of the food. The animal thus obtained 16.6 g nitrogen. The temperature rose rapidly from 38.1° to 41.1°. This is in accordance with earlier observations on particularly high temperature increases after fever-producing substances have been administered to a fasting animal simultaneously with a supply of food[1]). Unfortunately the

1) Krehl and Matthes, Arch. exp. Path. Pharmak., Vol 36, p 436.

sheep consumed but little food on the following two days and looked very sick. 75 g lactose were, therefore, injected subcutaneously. Although the sugar was excreted in the urine almost completely on the day of the injection and the two following days, the condition of the sheep improved at once after the injection: it resumed the consumption of food.

Curve 3.



1 -- Date; 2 -- Temperature.

As a result of the animal's aversion to cottonseed flour this had to be replaced by 250 g hay on the 6th day and 7th day. In the 'after-period' lasting for three days the animal consumed its fodder leaving no trace.

During the first six days of the fever period the sheep excreted 5.7 g nitrogen in the urine daily. This is 0.4 g more than during his fast, even though nitrogen absorption was about 6 g daily. According to our experience with this animal, this quantity of nitrogen at the caloric value of the food (1,186 calories) was insufficient to maintain the animal in nitrogen equilibrium. If, nevertheless, there was no protein loss during the fever period this must be attributed to the combined effect of lactose administration and the tendency of the convalescing organism after a period of hunger, to retain protein-like substances.

In experiments 1 and 3 similar caloric food values are found for the febrile periods per kilo of body weight. In experiment No 3 the amount of calories and the quantity of nitrogen resorbed are somewhat lower. Nevertheless the organism puts on nitrogen in this experiment while nitrogen is lost in experiment No 1. The 'gain' during the fever in experiment No 3 is only insignificantly smaller than that of the 'after-period' in experiment No 1, where the same amount of calories and slightly more nitrogen are taken up per kilo.

Since the organism strives to replenish the nitrogen lost during hunger, it prevailed even over the influence of the fever on protein metabolism.

Summarizing the results we find that an animal loses proteins during an acute attack of fever even while its food consumption would suffice to maintain nitrogen and energy exchange in equilibrium in a healthy individual.

Where fever was induced in an animal in a state of considerable protein gain and large amounts of proteins and carbohydrates were fed during this period, it was possible to maintain the 'gain' throughout the entire febrile period. Similarly it is possible to induce a starved animal to lay down protein during a febrile period by ample feeding during this time.

Is the restriction of protein expenditure by carbohydrates in such cases -- and this seems to be the salient point -- of the same magnitude as it would be without the febrile condition? A reply to this question would be simple if the animal could be induced easily to consume the same amounts of food during a fever attack than it did before. If such were the case one could add carbohydrates to the food during fever, and the resultant nitrogen excretion would enable us to judge whether an equilibrium can be maintained or not. This ideal experimental arrangement did not obtain in our experiments. We cannot make any exact statements on the magnitude of the protective effect of lactose administration. We have shown above, based on the results of experiment No 2, that on the one hand the sugar had a reducing effect on protein metabolism -- or, to put it more cautiously, on nitrogen excretion. Furthermore, however, we showed that in spite of this and despite nitrogen retention during fever, protein breakdown was greater than normal. This was made evident in the 'after-period' which demonstrates metabolic changes during convalescence: despite omission of carbohydrates, nitrogen gain was considerably enhanced, through the increase in protein supply was insignificant. von Noorden remarked upon the fact that increased protein breakdown can be concealed behind nitrogen retention. This is doubtless correct. We must always remember that present methods investigating metabolism only elicit information on the difference between substances taken up by the body and those excreted by it. Naturally this places on us the burden of extreme caution in interpreting the results.

EXCRETION OF PHOSPHORIC ACID

In experiments 2 and 3 we have investigated the excretion of phosphoric acid in relation to uptake. As we know, under normal feeding conditions, ruminants excrete the major portion of phosphoric acid by way of their intestines, and only very little in their urine. This mode of excretion does not permit any conclusions as to what part of the phosphoric acid excreted

with the feces is due to undigested food substances. We can only find out whether any phosphoric acid has been retained or whether a loss has occurred -- resorption values cannot be obtained.

In experiment No 2 we only investigated the urine during the fever -- and the 'post-experimental period' respectively. Numerous analyses by other authors have shown that a sheep fed raw fodder excretes an average of 0.02-0.03 g P_2O_5 daily under normal conditions. During the fever period an average of 0.061, was excreted daily with the urine and during the 'after-period' 0.135 g. During both these periods, therefore, phosphoric acid excretion is markedly increased compared to the figures for normal feeding mentioned above. As we will show below in experiment No 4 this excessive excretion is a result of the choice of fodder. For the moment it will suffice that the phosphoric acid content of urine is lower during fever than it is during the 'after-period'. This finding alone does not, however, permit any conclusions regarding the extent to which phosphorus compounds are broken down in the body, because, as we have mentioned above, the major portion of phosphoric acid is excreted with the feces. During fever relatively larger amounts of phosphoric acid are excreted by this route than during the preceding period. P_2O_5 uptake during the fever was reduced by 15.4 g, but only 5.6 g P_2O_5 less were excreted with the feces. This would indicate a significant relative increase in phosphoric acid excretion if we knew how large a portion of this stems from phosphoric acid of the food which has not been absorbed. On the one hand it is unlikely that phosphoric acid absorption during fever should deviate significantly from the norm. There is no reason whatever for such an assumption. But on the other hand we cannot fully determine the portion of phosphoric acid from the preceding period which is still contained in and analyzed with the feces of the f brile period.

Taking these circumstances into consideration it seems plausible that despite the gain of nitrogenous substances which took place during fever in experiment No 2 some phosphoric acid was lost.

During the 'post-experimental period' phosphoric acid replenishment took place despite increased urinary phosphoric acid excretion.

Conditions governing phosphoric acid excretion are even more complicated in experiment No 3 where a 10-day starvation period preceded the fever.

Henneberg[1] has remarked that herbivorous animals become carnivorous during a period of hunger, i.e., they excrete phosphoric acid synthesized in the body through the urine. This was confirmed in our case. The urine contained increasing, and finally very considerable, amounts of phosphoric acid. At the same time the content of this acid in the feces decreased, and -- this is worthy of note -- it even decreased percentually.

It is obvious that we cannot expect to find as much phosphoric acid in 22 g feces of the last fasting days as we did in 600 g. But we should have expected a percentual increase to the point where the content of the intestines would -- so to speak -- be saturated with phosphoric acid, i.e., as much P_2O_5 would be bound to alkalis and alkaline earths as these are able to bind in the intestines. This should have been the case if the intestines had the specific function of phosphoric acid excretion. At the start of the starvation experiment we found 2.1% P_2O_5, during the last few days 1.2%, and after one day of refeeding again 2.4%!

Two interpretations for this striking behavior come to mind. First, it is possible that the alkaline earths present in the intestines were strongly reduced[2]. If these were reduced by 50% they would just suffice to bind the phosphoric acid of the 10th day of starvation. But such an assumption is rather improbable[3], the more so since the alkaline earth content two days later was sufficient to bind much larger quantities of phosphoric acid. Furthermore, we must imagine that blood flow to the intestines is much reduced during starvation, and is much less than normal, as a result of reduced glandular activity, peristalsis and absorption, and because the intestines are partly relieved of their content and collapsed. A reduced blood supply then leads to reduced excretory activity. The total quantity of excreted phosphoric acid increases daily, nitrogen excretion decreases (wasting of bones; see hunger experiments on Cetti).

Ordinarily the normal mode of excretion is gradually resumed when the starvation period is terminated and normal feeding reintroduced. This was not the case in the present experiment. The urine is not gradually depleted of phosphoric acid, while its content in the feces increases; at first both urine and feces increase their phosphoric acid content. An explanation

1) J. f. Landwirtschaft, 1865. Feeding experiments of Salzmunde. A critical appreciation by W. Henneberg.
2) Cf. Rey, On excretion and resorption of lime. Arch. exp. Path., Vol 35, 295-305.
3) F. Müller, Zschr. f. Biol., Vol 20, 327-377.

for this phenomenon must be found either in the quality of the fodder or in the febrile condition. It is unlikely that the fever caused this considerable excess excretion[1]). At any rate no such increase in phosphoric acid excretion unaccompanied or followed by an increase in nitrogen in the urine has been observed. It seems much more plausible to relate this phenomenon to the excretion of ample amounts of P_2O_5 ingested with the feed. It is striking though that the urine contains the major portion.

After increasing significantly at the start, phosphoric acid values of the urine then decrease, without however reaching the normal low values by the last day of observation. The percentual content of P_2O_5 in the feces rises at first, as has been mentioned (up to 3.9% of dry feces). On the last day of cottonseed flour feeding 18.9 g P_2O_5 of the 19.4 g ingested are excreted.

After hay and oat feeding is introduced during the last days of the fever period, the percentual values of the content found in the feces return to normal. At the end of the experiment 38.2 g P_2O_5 out of the 46.9 g in the feed, appear in the excrements. A possible phosphoric acid loss of the body can therefore be excluded for this period.

The following experiment was conducted to examine the assumed influence of the feed on the mode of excretion of phosphoric acid.

Experiment No 4.

After posing the question: can we induce excretion of phosphoric acid through the kidneys by feeding a phosphorus-rich and calcium-poor diet, such as for instance, cottonseed flour? -- the experimental arrangement is simple.

After pre-feeding for some time with unspecified amounts of hay, the sheep was fed cottonseed flour exclusively for 10 days, and thereafter again 600 g hay (8.5 g N) each day were fed for a further 10 days. The excrements were analyzed in the usual manner throughout this entire span of time.

Since the animal could not be induced to consume all of the 500 g flour offered daily, an average of 232 g with 16.6 g nitrogen only were ingested daily during this period. The entire amount of hay offered was consumed.

1) Compare the investigations by Zuelzer, Virch. Arch., Vol 66, p 223. Lowit, Lectures on General Pathology, Jena, 1897. Dmitriewsky, Arch. int. Pharmacodyn., 1901.

As the table below shows an acid urine containing increasing quantities of P_2O_5 was excreted during the first period. Thus the flour had the same effect as animal fodder.

Table IV. -- Experiment 4.

(1) Datum	(2) Körpergewicht	(3) Einnahmen (4) Nahrungsmittel	(5) darin P_2O_5	(6) Trinkwasser	(7) Harnmenge	(8) Spec. Gew.	Ausgaben (9) Kotmenge	(10) Harn P_2O_5 g	(11) Kotin Proc.	(12) (13) Kot P_2O_5 g
1901		(14)								
(15) 5. I	600 g	Heu	2,052	800	700+	1025	145,5	0,923	1,596	1,573
Baumwollsamenmehlperiode.										
16. I 33500	500 c	Bier			0	656+	1034	262,9		
17.	350 •				600	360—	1030	163,3		
18.	500 •				400	425 —	1027	142,5	1,159	1,910 6,326
19.	127 •				500	530—	1025	150,0		
20.	100 •				800	515 —	1035	99,0		
(16) In Summa	1577 g	Bier	10,53	2300	2180			508,6	3,190	8,325
(17) Tägl. Durchschn.	315 •	(30)	5,11	460	436	1030	101,7	0,638	1,910	1,666
21. I 33400	150 g	Bier			900	390—	1030	101,95		
22.	100 •	(30)			1000	430—	1020	46,1	1,013	3,303 3,472
23.	150 •				100	355—	1025	63,67		
24.	140 •				0	250—	1021	109,3	1,158	
25.	240 •	(30)			510	240—	1040	57,2		3,530 2,377
(18) In Summa	740 g	Bier	15,02	2510	1055			378,22	10,786	5,849
(19) Tägl. Durchschn.	145 •	•	3,504	502	331—	1028	75,64	2,157	3,417	1,170
10.—25. I		(30)								
(20) Summa	2017 g	Bier	59,95	4800	4135		1186,8	13,976		14,177
(21) Tägl. Durchschn.	232 •	•	5,05	480	413	1029	118,7	1,398	2,063	1,416
(22) Heuperiode.		(23)								
26. I	600 g	Heu		800	220—	1020	122,43	0,935		
27.	600 •	•		1200	350—	1029	82,27		4,362	8,965
28.	600 •	•		900	520—	1025	165,02	0,409		
29.	600 •	•		900	310—	1030	259,1			
30.	600 •	•		900	415—	1032	375,25			
(24) In Summa	3600 g	Heu (23)	4,10	4300	1815		1394,07	2,1138		17,173
(25) Tägl. Durchschn.	600 •	•	2,05	860	363	1027	235,6	0,423	3,33	3,435
31. I	600 g	Heu (23)		1200	600+	1020	490,9		1,785	28,655
1. II	600 •	•		900	480+	1033	398,6			
2.	600 •	•		600	450+	1035	391,5			
3.	600 •	•		900	830+	1020	426,95			
4.	600 •	•		900	660+	1027	291,1	0,0165		
(26) In Summa	3600 g	Heu (23)	4,10	4500	3020		1798,95	0,0925		20,470
(27) Tägl. Durchschn.	600 •	•	2,05	750	604	1030	399,8	10,085	1,785	4,094
(28) 31. I—4. II										
Summa	6000 g	Heu (23)	8,20	8100	4835		3192,0	2,206		37,643
(29) Tägl. Durchschn.	600 •	•	2,05	810	480	1028	310,2	0,221	2,56	3,764

1) In 100 cm3 amounts which cannot be weighed (urine made up to 1000).
2) In 150 cm3 amounts which cannot be weighed (urine made up to 1000).

(-- Date; 2 -- Body weight; 3 -- Uptake; 4 -- Feed; 5 -- P_2O_5 content; 6 -- Drinking water; 7 -- Amount of urine; 8 -- Specific weight; 9 -- Excretion; 10 -- Amount of feces; 11 -- Urine-

P_2O_5 g; 12 -- Feces %; 13 -- P_2O_5 g; 14 -- Hay; 15 -- Cottonseed flour period; 16 -- Total; 17 -- Daily average; 18 -- Total; 19 -- Daily average; 20 -- Total; 21 -- Daily average; 22 -- Hay period; 23 -- Hay; 24 -- Total; 25 -- Daily average; 26 -- Total; 27 -- Daily average; 28 -- Total; 29 -- Daily average; 30 -- Cottonseed flour.

During the subsequent hay period the phosphoric acid content of the urine returned to its normal low values. The reaction becomes strongly alkaline again, and the hippuric acid which had disappeared almost completely during cottonseed flour feeding, recurs in large amounts when urine is left with HCl.

As I have only found out after completion of my last experiment, Bertram, feeding goats with gluten learned that the phosphoric acid content of urine could rise up to 1.4 g per day. The reaction remained strongly alkaline. In addition to gluten 800 g hay were fed daily which explains the reaction. Exclusive feeding with cottonseed flour in our case understandably made the urine acid due to the absence of vegetable acid alkalis. The reaction is thus neither the cause nor a consequence of the presence of larger quantities of phosphoric acid in the urine of herbivorous animals. Bertram extended his experiments[1] feeding K_2PHO_4 with hay fodder. After this approximately 1 g P_2O_5 appeared in the urine which remained constantly alkaline. Simultaneous feeding of calcium then led to a return of the phosphoric acid values to normal, but the experimental arrangement was not flawless (compare, for instance Tereg and Arnold in Pflug. Arch., Vol 32, pp 122-176). These two authors conducted similar experiments in dogs but, unfortunately, they gave no indication of Mg values in their experiments. While these experiments indicate that the alkaline earth content of the intestines is very important for the phosphoric acid excretion values, we must nevertheless admit -- according to the results of the starvation period in experiment No 3 -- that other factors are present.

With regard to the fever experiment No 3 we conclude, as a result of findings in experiment No 4, that the high phosphoric acid values in the urine on the third and fourth day of experiment No 3's febrile period, were caused by the cottonseed flour feeding.

Analogously to the percentual increase in the P_2O_5 content of the feces during the first days of fever mentioned before, this demonstrates a rapid absorption and metabolism of the ingested food. In experiment No 2 phosphoric acid appears first

1) Zschr. f. Biol., 1878, Vol 14, p 335.

just as rapidly in the feces. Between 15-21 June (compare Table II) 6.2 P_2O_5 were ingested with the feed. The feces for the period 14-18 June contained 1.4% or 20.2 g P_2O_5. Beginning on 22 June, 11.32 g P_2O_5 were fed. The feces accumulated during the period 19-23 June (which only contained 2/5 of the feces of the new feedings) contained 1.8% or 26.6 g P_2O_5; i.e., a considerably increased phosphoric acid excretion already after only two days. (Nitrogen analysis of the feces yielded the information that the increase in phosphoric acid did not stem from unabsorbed food portions).

Regarding the influence exerted by the fever on the phosphoric acid metabolism in experiments No 3 we can only say that phosphoric acid retention set in at the end of the febrile period continuing to the end of the experiment.

Dmitriewsky[1] in his extensive investigations of various fever-producing infections, did not find an increase in phosphoric acid excretion during the febrile period. On the contrary at times he found it to be decreased. Our experiment No 2 does not however agree with these findings.

APPENDIX AND DOCUMENTATION

Experiment No 1.

400 g hay of medium quality and 400 g rye bran daily which were fed continuously from 27 Jan 1900 on, were found to be an average maintenance diet. Figures for nitrogen uptake given in Tables I-IV have been obtained by subtracting the N in the residues of the fodder from that of the total fodder offered. The residues were dried, pulverized and analyzed for N, as was the fodder itself.

The pre-febrile period, main period and 'after period' respectively were subdivided into several smaller sections: Periods I-V, VI-X, XI-XIII. The febrile period was subdivided according to the behavior of body temperature. The average figure for absorption during fever is depressed by period VI. Since this figure is partly incorrect due to the nitrogen in feces which stems -- in part -- still from the period of greater food consumption, thus showing N values which are too high, we can neglect it. We then obtain an absorption value for periods VII-X which almost equals that of the pre-febrile period.

To produce fever the animal was given a total of 47.5 cm^3 mucus toxin in nine subcutaneous injections over the period 14 Feb to 3 March 1900.

1) Arch. Int. Pharmacodyn., 1901, No 2.

Table I -- Experiment 1.

(1) Datum	(2) Gewicht	(3) Einnahmen (4) Futter	N	(5) Harnmenge	(6) Kotmenge	Ausgaben (7) Harn (8) N	Kot (9) N	N Bilanz (10)	
I. Periode.		(11)							
21. I	36,1 kg	Zugewogen (gl.:		420	702,2	12,50			
22.		400 Heu und		642	785,2	11,47			
23.		500 Roggenkleie		395	735,6	9,2N	21.– 25. I		
24.		1500 ccm		680	679,0	7,47	2,072 Proc.		
25.		Wasser		1055	669,4	10,50	= 29,40 g N		
26.				815	573,0	8,71			(19)
			16,12*	666	690,7	9,04	5,89	+0,29	*) Mit Berück-
II. Periode.		(12)							sichtigung des in
27. I		Zugewogen (gl.		915	614,1	10,22			den Rückständen
28		400 Heu und		930	695,9	9,63	26.– 30. I		gefundenen
29.		400 Roggenkleie		825	657,0	9,53	2,254 Proc.		Stickstoffes.
30.		1500 ccm		810	749,0	9,80	= 29,01 g N		
31.	36,5 kg	Wasser		710	700,8	9,60			
			14,46*	844	694,5	9,76	5,79	−1,09	
III. Periode.		(13)							
1. II				510	656,0	7,28			
2.		Wie in voriger		670	681,5	8,29	31. I—4. II		
3.		Periode		760	809,0	12,04	2,039 Proc.		
4.				820	743,0	9,60	= 26,27 g N		
			15,25*	688	700,9	9,54	5,25	+0,47	
IV. Periode.									
5. II		(14)		580	762,0	7,52			
6.				840	843,7	9,72	5.—9. II		
7.		Desgleichen		900	770,2	10,01	2,072 Proc.		
8.	36,3 kg			810	913,0	10,22	= 27,02 g N		
			15,42*	793	822,2	9,58	5,41	+0,05	
V. Periode.									
9. II		(15)		420	807,7	7,21	10. II		
10.				*	894,2	*	5,67 g N		
11.		Desgleichen		410	737,5	9,01	11.—15. II		
12.				510	944,9	10,01	2,030 Proc.		
13.				400	848,3	7,95	= 24,95 g N		
		(16)	15,62*	435	810,1	8,03	5,21	+1,78	
VI. Periode.		Fieberperiode. (VI—X).							
14. II	36,2 kg	Zugewogen (gl.		1700	819,7	12,85	16.—20. II		
15.		400 Heu und		760	769,0	8,08	1,900 Proc.		
16.		400 Kleie		510	630,1	6,48	= 22,57 g N		
		1500 ccm Wasser	11,10*	927	741,6	9,34	4,84	−3,07	
VII. Periode.		(17)							(20)
17. II				480	768,0	9,07			**) Die ange-
18.		Desgleichen		430	631,6	8,15			gebenen Wasser-
19.		1600 Wasser**		370	481,3	8,09			quanta sind
			12,07*	427	626,9	8,44	4,51	−0,78	Mittelwerthe der
VIII. Periode.		(18)							in den betr.
20. II				600	703,8	11,25	21.– 25. II		Perioden (gl ge-
21.		Desgleichen		420	674,1	6,86	2,030 Proc.		trunkenen Mengen
22.		1300 Wasser		370	641,5	9,11	= 22,14 g N		
			13,19*		673,2	9,07	4,46	−0,53	

1 -- Date; 2 -- Weight; 3 -- Uptake; 4 -- Fodder; 6 -- Quantity of urine; 6 -- Quantity of feces; 7 -- Excretion; 8 -- Urine N; 9 -- Feces N; 10 -- N balance; 11 - Weighed out daily 400 g hay and 500 rye bran, 1500 cm3 water; 12 -- Weighed out daily 400 g hay and 400 g rye bran, 1500 cm3 water; 13 -- As in preceding period; 14 -- the same; 15 -- the same; 16 -- Fever period: weighed out daily 400 g hay and 400 g bran, 1500 cm3 water; 17 -- the same, 1600 cm3 water; 18 -- the same, 1300 cm3 water; 19 -- *Taking into consideration the quantity of N found in the residues; 20 -- **The quantities of water given are average values of the water drunk in the respective periods; Periode = period.

Table I (Concluded)

(1) Tag	(2) Gewicht	(3) Einnahmen (4) Futter	N	(5) Harn	(6) Kot	(7) Ausgabe Harn N (8)	Kot N (9)	N Bilanz (10)	
IX. Periode. (11)									
20. II	35,2 kg	Zugewogen 1 g		1095	677,1	10,58			
21.		400 Heu und		570	680,4	8,48			
22.		400 Kleie		510	578,3	8,07			
23.		1300 ccm Wasser		440	643,1	10,08			
			11,87*	585	647,5	9,30	4,34	—1,78	
X. Periode.									
27. II				570	813,0	8,44	26. II		
28.		(12)		680	626,0	8,15	— 2. III		
1. III		Desgleichen		170	312,4	8,61	1,305 Proc.	(17)	
2.		1100 Wasser		410	514,8	7,56	= 20,34 g N		
3.				150	185,5	7,76	3.—7. III		
4.				280	386,6	8,57	1,570 Proc.		
5.				390	370,1	7,69	= 18,11 g N		
		(13)	11,76*	466	476,9	7,65	3,59	—0,94	
XI. Periode. Nachperiode.									
6. III	33,7 kg			300	520,1	7,00			
7.				545	524,4	9,83			
8.		(14)		600	452,1	8,80			
9.		Desgleichen		1170	580,6	4,58	8.—13. III		
10.		1400 Wasser		480	443,0	4,84	1,736 Proc.	(17)	
11.				450	460,0	6,02	= 25,75 g N		
12.				540	574,8	6,80			
13.				500	655,5	7,10			
			12,58*	617	527,1	6,52	4,12	+1,94	
XII. Periode.		(15)							
14. III	34,5 kg			580	508,4	6,58			
15.		300 Heu		520	516,7	6,05	14.—18. III		
16.		250 Kleie		400	678,3	6,16	1,568 Proc.	(17)	
17.		1100 Wasser		310	673,1	5,75	= 19,25 g N		
18.				270	550,3	5,81			
			10,81	416	605,4	6,19	3,85	+0,77	
XIII. Periode.		(16)							
19. III		300 Heu		360	645,6	6,13			
20.		250 Kleie		400	508,7	6,67	19.—23. III		
21.		verzehrt		270	584,1	6,10	2,170 Proc.	(17)	
22.		1100 Wasser		230	652,8	6,28	= 20,17 g N		
23.	34,7 kg			210	721,5	6,58			
			11,39	312	662,5	6,34	4,03	+1,04	

1 -- Date; 2 -- Weight; 3 -- Uptake; 4 -- Fodder; 5 -- Quantity of urine; 6 -- Quantity of feces; 7 -- Excretions; 8 -- Urinary N; 9 -- Fecal N; 10 -- N balance; 11 -- Weighed out daily 400 hay and 400 bran, 1300 cm3 water; 12 -- the same, 1100 water; 13 -- 'Post-experimental (after) period'; 14 -- the same, 1400 water; 15 -- 300 hay, 250 bran, 1100 water; 16 -- 300 hay, 250 bran eaten, 1400 water; 17 -- %; 18 -- *Taking into consideration N found in residues; Periode = period.

Average Daily Values of Pre-Exp. Period, Main Period, and 'After-Period'

(1) Nahrung	N	(2) Futteraufnahme	(3) Harnmenge	(4) Harn-Spez. Gew.	(5) Kotmenge	(6) Kot Trockensubstanz	(7) Harn N	(8) Harn N Resorbiert	(9) Kot N	(10) N Bilanz
(12) Vorperioden										
7. I—13. II	15,17	1506	653	1020	772,8	34,2 Proc.	9,31	5,42	61,1 Proc.	+0,44
(13) Hauptperioden										
14. II—5. III	11,76	1200	564	1034	662,7	37,6 "	6,56	4,30	63,2 "	1,10
(14) Nachperioden										
6. III—28. III	11,76	1200	472	1033	595,9	37,1 "	6,38	4,02	63,6 "	+1,36

1 -- N in food; 2 -- Water uptake; 3 -- Quantity of urine; 4 -- Urine -- specific weight; 5 -- Quantity of feces; 6 -- Feces -- dry substances; 7 -- Urinary N; 8 -- Fecal N; 9 -- N absorbed; 10 -- N balance; 11 -- %; 12 -- Pre-experimental period; 13 -- Main periods; 14 -- After periods.

Resorption of Raw Food Protein

(1) Periode	(2) Vorperiode I	II	III	IV	V	II–V	(3) Fieberperiode VI	VII	VIII	IX	X	VI–X	(4) Nachperiode XI	XII	XIII	XI–XIII
(5) N der Nahrung	16,1	11,5	15,2	15,4	15,6	15,2	11,1	12,7	13,2	11,9	11,6	11,8	12,6	10,8	11,1	11,8
(6) N im Kot	5,9	5,8	5,2	5,4	5,2	5,4	4,8	4,8	4,5	4,5	5,0	4,3	4,1	3,8	4,0	4,0
(7) Resorbiert N	10,2	5,7	10,0	10,0	10,4	9,8	6,3	7,9	8,7	7,6	6,6	7,5	8,5	7,0	7,1	7,8
(8) In Prozenten	63,5	50,0	65,5	65,0	66,6	64,1	56,5	64,2	66,1	63,8	61,5	63,8	67,1	64,4	64,0	66,6

1 -- Period; 2 -- Pre-experimental period; 3 -- Fever period; 4 -- After-period; 5 -- N in feed; 6 -- N in feces; 7 -- N resorbed; 8 -- In %.

The first injection of 2 cc induced a moderate temperature rise to a maximum of 40.1° (compare Curve 1), the effect lasting approx. two days. The second injection resulted in an insignificant increase in temperature. Therefore the dose of the third injection was increased to 5 cc and had approximately the same effect as the first. When the dose was increased to 10 cc rather high temperatures lasting for 60 hours were obtained. Finally, injections were given daily and 28.5 cc toxin were used. During this period continuous remittent temperatures were obtained. The highest peaks could be observed about six hours after the injection. During the after-period starting on 6 March, temperatures were normal at all times.

Experiment No 2

The sheep was placed in the experimental stable on 7 June and fed 400 g hay and 500 g oats daily. All of this was consumed. Nitrogen determination was started on 15 June. After seven days of observation 200 g cottonseed flour was added to the feed, raising the daily nitrogen supply from 16.8 to 31.1 g. In the course of 11 days the animal adapted to these large quantities and excreted approximately constant amounts of N. It seemed desirable to examine these processes to gain insight into the resorption conditions with the chosen enriched feed and nitrogen gain caused by it. Resorption figures for the various sub-periods are given below:

(1) Perioden	I	II	III	IV	V
(2) N der Nahrung	16,8	31,1	31,1	24,3	27,7
(3) N im Kot	5,0	6,5	7,7	7,3	6,8
(4) Resorbiert N	11,8	24,6	23,4	17,0	20,9
(5) Prozente	70,2	79,0	75,2	69,8	75,4

1 -- Periods; 2 -- N of feed; 3 -- N in feces; 4 -- Resorbed N; 5 -- In %.

Of 31.1 g nitrogen taken up daily 75.2% was thus resorbed. Comparing this figure with period I it can be calculated that 81.1% of the cottonseed flour was utilized. Repletion figures are given in Table II.

100 g lactose was added during the fever period in order to administer a sugar which is not very sweet, readily dissolved in water, and has no stimulating effect on the intestines.

Temperatures during the febrile period were sufficiently high for a fever experiment. The first reaction to an injection of 6 cc was a rise in temperature by 1.3° which reached its peak within two hours. The temperature did not rise as a result of the second injection. Only the third injection again led to a period of fever lasting three days. 11 cc of toxin was used for the six-day fever period. Just as in the case during the first experiment no disease symptoms other than temperature rise and diminished appetite were observed in the animal during the febrile period.

The considerable amount of 6.9 g N put on daily, which was observed during the pre-febrile period (28 June-3 July 1900), is a very high figure for an adult sheep. It has frequently been doubted that adult sheep can be fattened with meat. But the extensive investigations by Pfeiffer and Kalb[1]) have confirmed that this is possible. In a 100 day fattening experiment the authors demonstrated a continuous, not insignificant nitrogen gain. The difference in fodder used does not, however, permit a direct comparison between these authors' results and our own.

In order to decide the question whether despite the positive N balance during the fever period, the protein breakdown were larger than in the normal state, the following should be considered. If we assume at first a proportional relation between N uptake and excretion, we obtain a figure of +6.4 g N put on daily when 23.4 g are fed -- based on the degradation values during fever. In the normal state +6.9 g were found.

1) Landwirthschaftl. Jahrb., 1892, pp 175-209.

Table II - Experiment 2

(1) Datum	(2) Gewicht	(3) Einnahmen	N	P₂O₅	(4) Wasser	(5) Harnmenge	(6) Kot	NH₃	(14) Harn N	(13) Ausgaben P₂O₅	(15) Kot N	(15) Kot P₂O₅	(16) Bilanz N
15. VI	38 kg	(7) Täglich verzehrt 400 g Heu + 500 g Hafer	16,8	6,17	1500	250	[illegible]		5,98		14.–19. VI 1,628 Proc. = 26,35 g N (18)	14.–18. VI 1,492 Proc. = 20,19 g P_2O_5 (18)	
16.						310	[illegible]		6,04				
17.						290	71		5,71				
18.						400	[illegible]		6,12				
19.						330	[illegible]		6,06		19.–23. VI 1,650 Proc. = 24,76 g N	19.–23. VI 1,540 Proc. = 26,62 g P_2O_5	
20.						310	64		6,22				
21.	38,4 kg					415	50		6,30				
			16,8	6,17	1500	345	[illegible]		6,20		5,01	4,59	+5,[illegible]
22.		(8) Täglich verzehrt 400 g Heu, 500 g Hafer, 200 g Baumwollsamenmehl	31,1	11,32	1500	410	[illegible]		9,10				
23.						510	[illegible]		12,34				
24.						460	[illegible]		12,19				
25.						430	[illegible]		13,87				
26.						435	91		14,81		24.–28. VI 2,210 Proc. = 57,15 g N (18)	24.–28. VI 2,917 Proc. = 49,54 g P_2O_5 (18)	
27.						610	101		16,77				
			31,1	11,32	1500	461	[illegible]		13,11		6,01	6,55	+11,4
28.		(9) Gleiche Nahrung vom 22.–27. VI	31,1	11,32	1500	480	91		15,50				
29.						560	104	(17) Harn vom 2.+3. VII 2201,10 [illegible] NH₃	17,23				
30.						600	[illegible]		16,20		29. VI–3. VII 2,240 Proc. = 38,83 g N (18)	29. VI–3. VII 2,510 Proc. = 48,71 g P_2O_5 (18)	
1. VII						530	[illegible]		17,52				
2.						710	[illegible]		16,11				
3.						500	[illegible]		15,62				
			31,1	11,32	1500	563	[illegible]	(17a)	[illegible]		7,71	2,75	+7,0
(10) Fieberperiode													
4.	40,6 kg	(11) Zugewogen täglich wie vom 22.–27. VI dazu 50 g Stärke im Trinkwasser 100 g Milchzucker	24,3*	8,55*	2500	1100	92	5.–7. VII [illegible]121 g, 0,314	18,13	4.–9. VII 0,309	4.–8. VII 2,110 Proc. = 37,17 g N (18)	4.–8. VII 2,545 Proc. = 44,58 g P_2O_5 (18)	
5.					2200	730	[illegible]		11,77				
6.					2000	310	[illegible]		[illegible]				
7.					2500	460	[illegible]	8.–10. VII 1377 g, 0,459	13,33				
8.					2200	380	[illegible]		8,00				
9.					2000	290	[illegible]		9,42				
			24,3	8,55	2150	600	95	0,372	12,32	0,061	7,21	8,53	+4,7
10.	40,4 kg	(12) Zugewogen wie vom 22.–27. VI	31,1	11,32	2200	1040	76	11.–13. VII 0,825 tgl. 0,275	11,54	10.–14. VII 0,676	9.–14. VII 2,290 Proc. = 40,55 g N (18)	9.–14. VII 2,716 Proc. = 45,55 g P_2O_5 (18)	
11.					2500	600	71		11,06				
12.					2000	660	66		12,71				
13.			24,4*	8,87*	2500	620	96	14.–15. VII 0,918 tgl. 0,159	15,40				
14.					2000	630	80		14,65				
15.	40,5 kg				1800	680	91		14,47				
			27,7	10,10	2180	730	82	0,367	13,12	0,135	6,51	8,09	+7,5

1. Date; 2. Weight; 3. Uptake; 4. Water; 5. Amount of urine; 6. Amount of feces; 7. Daily amounts eater: 400 g hay, 500 g oats, 200 g cottonseed flour; 9. same food as during the period 22-27 June; 10. Fever period; 11. Weighed out daily as during 22-27 June, additionally 50 g starch; in drinking water 100 g lactose; 12. Weighed out as during period 22-27 June; 13. Excretions; 14. Urine; 15. Feces; 16. Balance; 17 Urine from .....; 17a. Daily; 18. %.

*) Taking into consideration the N and P_2O_5 values found in the residues.

Taking normal conditions as our starting point we can calculate that + 5 g N daily are put on at an uptake of 17 g. The value found during fever was + 4.7 g. The assumptions made here are not correct inasmuch as repletion increases relatively little with increased supply, the figure of + 6.4 g calculation for gain is too high, and the daily gain during fever would in fact be less. As against this our second hypothetical figure of + 5.0 g N put on at an uptake of 17 g under normal conditions, is too low, because we have found a gain of 5.6 g daily (15-21 June) where only 11.7 g N were resorbed. At that the effect of carbohydrate supply has been totally disregarded in this calculation.

Experiment No. 3.

Pre-febrile period.

The sheep was placed in the experimental stable on 15 September and collection of excrements was started on 19 September. Fodder consisting of 450 g oats and 250 g hay daily was consumed without leaving a trace.

Utilization of 54.4% of raw protein of which only 5.5 g N were resorbed, did not permit a comparison with the fever

period of experiment No 1 as had been intended. Resorption values are the lowest observed in any experiment:

At	10.1 g	N in the feed	Experiment	2	resorbs:	54.4%.
"	15.2 "	" "	"	1	"	64.1%.
"	16.8 "	" "	"	2	"	70.2%.
"	31.1 "	" "	"	2	"	75.2%.

The astonishingly slight resorption during the period under consideration cannot be attributed to the fodder mixture. According to the results of the Hohenheimer feeding experiments no depression of the digestibility of raw proteins should occur with a mixture of 400 g hay plus 500 g oats, and 250 g hay and 450 g oats respectively. However, in the feeding experiments for agricultural purposes the quantities used were not as small as these. The possibility cannot be excluded that intestinal bacteria may remove approximately equal amounts of nitrogen for their own use when feed differing only quantitatively is supplied. They would thus use up relatively more nitrogen at lower protein supplies.

Fasting period. The animal excreted urine and feces daily during the 10 day fast, the latter in decreasing quantities. Dry content of the feces was increased. Nitrogen content of the urine and its abnormal behavior has been noted above. I have not been able to find an analogous case in the literature. Table III shows that urinary N was constant prior to the first day of starvation. The results during the first days of starvation are even more astonishing when we consider that the animal was kept undernourished prior the actual period of privation.

Initially increased ammonia excretion is gradually reduced during fasting. Relative increases, such as von Noorden reports, were not observed. During the pre-febrile period the ratio NH_3:N was 1:11.9, and on the last day of starvation it was 1:21.2. Thus there is also a relative reduction in ammonia in this experiment. Acetone, acetoacetic acid or albumin could not be demonstrated in the urine collected during the fast. But when urine mixed with crude hydrochloric acid and ferric-chloride was extracted with chloroform there was a distinct indicator reaction although the blue stain was not as strong during the last days of starvation. The reaction of the urine alkaline at first (or normal), became amphoteric but never distinctly acid. While approximately 2/5 of the water consumed during the pre-test period appeared in the urine, the quantity of urine during starvation was twice as much as the water taken up.

Fever period: 4-10 Oct. One hour before injection with mucus toxin the animal was fed for the first time after starvation with approximately 70 g cottonseed flour and 200 g hay. The remainder was given gradually so that total consumption was 150 g cottonseed flour and 400 g hay. 75 g lactose dissolved in the drinking water -- the entire amount of food and water was consumed. Temperatures taken every two hours (even at night) are shown in Curve 3.

75 g lactose dissolved in 750 water was infused subcutaneously taking antiseptic precautions on 6 Oct. We used a flat syringe directed towards the abdomen, injecting the upright standing restrained animal subcutaneously near the first lumbar vertebra. Sugar in the urine was determined by polarization.

We do not think that the temperature rise on 7 Oct was connected with the infusion. On 10 October the last temperature rise to 40.1° occurred, accompanied by a considerable increase in urinary N, ammonia and phosphoric acid.

Utilization of nutriments:

Fever period 4-10 Oct.

N in food	9.9 g
N in feces	1.13 g
N resorbed....	8.77 g or 88.5%.

'After-period' 11-13 Oct.

N in food	10.7 g
N in feces	6.19 g
N resorbed....	4.51 g or 42.1%.

These figures calculated directly on the basis of analyses on the respective days give only an inadequate picture of the true processes, because the feces collected did not stem exclusively from these fever days but, in part also from the preceding starvation period.

During starvation the animal kept its intestines as full as possible but, nevertheless, it lost some of its contents daily albeit in decreasing quantities. When feeding was resumed on 4 Oct, the newly formed feces remained at first to a large extent in the intestines and were only excreted after normal replenishment. Only seven days later normal quantities of feces were excreted. During the first days of resumption

of feeding starvation period feces appeared in larger quantities than before -- presumably because peristalsis was increased as a result of food consumption. The percentage of nitrogen rose only from the third day on significantly. This indicates that not only were starvation period feces excreted now but re idues of new food supplies were also intermingled with it. Possibly the entire amount of excrement during the last days of the fever period stemmed from feeding during this span of time. But they certainly represent only a part of the non-absorbed nutriments, while the other, more important part was only excreted after 11 Oct when quantities of the feces again reached normal proportions. Feces excreted during the 'after period' (11-13 Oct) had an abnormally high nitrogen content, again an indication of their being partially derived from food taken up during the febrile period.

5.8 g N was excreted in the feces between 4-8 Oct. According to the results obtained in agricultural feeding experiments 9.80 g N should have been expected. Thus at least 4 g N were retained unabsorbed in the intestines during those days. And this assumption does not take into consideration the possible depression of absorption by the sugar which was administered. During the next four days 21.18 g N, i.e., 4.24 g daily were excreted. Under favorable conditions of utilization and assuming the same pre-feeding, 3.05 g N should have been expected in the feces, or -- if the previously retained 4 g N were distributed evenly -- 3.85 g N daily. But if we correlate the amounts of N excreted during 9-13 Oct with the amounts excreted during the pre-test period, the 4.24 g N actually excreted compare with 4.8 g calculated. Thus the after-period would seem to offer more favorable conditions for resorption than the pre-test period.

Proceeding from the assumption that the febrile days account for the total quantity of feces excreted from 7 Oct (the first day with high N values) to 13 Oct, we can calculate an amount of 22.81 g N or 3.26 g fecal N daily. This would correspond to an absorption of 67%.

Nitrogen during the febrile and after-periods respectively, is also difficult to assess because the values for the feces are hard to estimate.

If, after what we have said about resorption of the nutriments, we assume that the quantity of nitrogen excreted between 7-13 Oct stems from the febrile period (certainly not too limited an assumption) the following calculation can be made regarding nitrogen turnover during the fever:

N in feed	9.9 g
N in feces	3.3 g
N resorbed	6.6 g
N in urine	6.4 g
N turnover +	0.2 g

This is a very slight gain. We tend to have greater faith in the low N values in the urine than in the hypothetical figures for the feces and we infer that the 'gain' was larger than that calculated above. No balance for the 'after-period' can be drawn up. It was too short to allow us to present normal values for the feces.

AMMONIA EXCRETION

Ammonia excretions in the urine were determined according to Schlosing's method in experiments No 2 and 3. No increase has been observed, rather a decrease of excretion in experiment No 3. As we know, however, in herbivorous animals the quantities of urinary ammonia cannot be used to gauge acid formation in the organism, since the acids are mostly excreted in a form firmly bound to alkalis. Nevertheless, it would have been possible that the body, impoverished in alkalis due to starvation, might have used ammonia for the binding of the possibly larger quantities of acids produced. But this was not the case. (On NH_3 excretion during fasting compare above.)

The methods used in our metabolism experiments in sheep followed closely the rules laid down for many years by Agricultural Experimental Stations. The animal was kept in a restraining stable of the Henneberg type and was equipped with a pouch for the collection of feces and a funnel for the urine. Proper treatment assured quantitative collection of the excrements as well as correct feeding, etc. Fecal pouches were changed daily; exactly 1/10 of the feces was preserved with chloroform in a glass jar. Except where daily examinations were required for special reasons, such average specimens were accumulated for five days in one bottle. Drying, etc., was done according to the usual methods. Fodder was examined according to the usual methods. Fodder was examined according to the techniques customary in Agricultural Experimental Stations. We were fortunate to enjoy the kind assistance of Prof. Dietrich-Marburg in these tests.

Table III - Experiment 3

[Legend on following page]

(1) Datum	(2) Gewicht	(3) Einnahmen (4) Futtermittel	(5) Darin N	Darin P_2O_5	(6) Trinkwasser	(7) Harn (8) Menge	(9) Harn Sp. G.	(26) Eiw. Vg.	Ausgaben (25) (27) Harn NH_3	Harn N	Harn P_2O_5	P_2O_5 : N	(28) Koth N Proc.	(29) Koth N g	Koth P_2O_5 %	Koth P_2O_5 g	(30) Bilanz N	Bilanz P_2O_5	(31) Bemerkungen
15. IX. 1900		450 g Hafer 250 g Heu	10,09	5,017	1000														Vom 15.—19. Sept. gleichmässige Vorfütterung.
19.		(10) dto. (11)	10,09	5,017	1000	350	1020	[illegible]	0,478	6,026	unwägbar						— 0,5		Koth vom 19. bis 23. IX als Durchschnittsprob analysirt. (32)
20.	39,9 kg	dto.	10,09	5,017	1000	300	1020	[illegible]	0,606	6,550							— 1,1		
21.		dto.	10,09	5,017	1000	400	1034	[illegible]	0,653	7,105			1,547	23,25	2.063	25,890	— 1,6	— 0,79	
22.		dto.	10,09	5,017	1000	450	1035	[illegible]	0,514	7,110							— 1,6		
23.		dto.	10,09	5,017	900	430	1038	[illegible]	0,547	7,045							— 1,5		
		tägl. Durchschnitt (12)	10,09	5,017	950	395	1029	[illegible]	0,566	6,779				4,60		5,178	— 1,3	— 0,16	
24.	39,8 kg	Hungerperiode (13)			200	420	1028	[illegible]	0,731	9,125	unwägb.		2,412	3,032	1,952	2,262			
25.					400	330	1024	[illegible]	0,512	8,096	0,0207	1 : 386	1,865	1,462	1,552	1,216			
26.					—	430	1020	[illegible]	0,489	7,560	0,235	1 : 32	1,918	1,051	1,184	0,682			
27.					400	195	1031	[illegible]	0,537	7,308	1,180	1 : 6,2	1,904	0,678	1,231	0,415			Der Urin zeigte während der ganzen Hungerperiode deutlich, gegen Ende an Intensität etwas abnehmende Indikanreaction (33)
28.					150	320	1022	[illegible]	0,595	7,140	1,658	1 : 4,3	2,224	0,794					
29.					100	320	1021	[illegible]	0,482	5,888	1,910	1 : 3,1	1,818	0,586	1,043	0,310			
30.					—	270	1024	[illegible]	0,344	5,723	2,583	1 : 2,2	2.200	0,381					
1. X.					50	290	1022	[illegible]	0,327	5,667	2,512	1 : 2,0	2,153	0,391	1,143	0,268			
2.					50	265	1025	[illegible]	0,274	5,326	2,789	1 : 1,9							
3.					150	260	1025	[illegible]	0,240	5,320	2,824	1 : 1,9	2,730	0,321	1,270	0,149			
			Summa		1470	3100			1,437	67,147	16,012			9,09		5,925			
			tägl. Durchschnitt		147,0	310	1024	[illegible]	0,454	6,715	1,601		2.139	0,909	1,339	0,593			enthielt nie Albumen, keine Reduction
4.	34,3 kg	(14) 400 g Hafer, 150 g Baumwollsaatmehl, 75 g Milchzucker	16,8	7,16	750	465	1015	[illegible]	0,368	4,124	2,583	1 : 1,6	2,170	1,020	1,217	0,576	+ 11,65	+ 4,3	Injection von 5 ccm Toxin (34)
5.		(15) 10 g Hafer, 5 g Bws, 37 g Milchzucker	0,5	—	270	500	1026	[illegible]	0,428	7,924	4,125	1 : 1,8	2,450	1,730	2,148	1,714	— 9,15	— 6,14	
6.		(16) 46 g Hafer, 79 g Bws, 75 g Zucker subcutan	6,3	2,45	—	550	1049	[illegible]	0,340	6,222	3,029	1 : 2,0	2,930	1,406	3,931	1,556	— 1,33	— 2,47	Um 6 h a. N. p. m. erhält das Thier 75 g Milchzucker in 750 aq. subcutan. (35)
7.		(17) 100 g Hafer, 50 g Zucker, 100 g Baumwollsaatmehl	9,7	3,5	250	330	1010	[illegible]	0,070	5,010	1,539	1 : 3,3	4,267	0,397			+ 3,26	+ 1,6	
8.		(18) 100 g Hafer, 200 g Baumwollsaatmehl, 150 g Zucker	15,8	6,0	750	300	1025	[illegible]	0,139	6,166	1,613	1 : 3,8	4,129	1,213	3,671	1,105	+ 8,4	+ 3,25	
9.	31,8 kg	500 g Hafer, 250 g Heu	10,7	5,5	1500	200	1025	[illegible]	0,119	4,788	0,364	1 : 13,2	3,300	0,638	1,961	0,379	+ 5,27	+ 4,5	
10.		(19) dto.	10,7	5,5	1100	700	1020	[illegible]	0,539	10,388	2,100	1 : 1,9	3,130	1,969	1,592	1,001	— 1,06	+ 2,4	
		(20) Summa	69,5	30,41	4620	3045			1,843	41,652	15,676			8,403		7,890	+ 16,45	+ 7,74	
		(21) tägl. Durchschnitt	9,9	4,34	660	435	1029	[illegible]	0,263	6,370	2,239		3,197	1,200	2,573	1,070	+ 2,35	+ 1,11	
11.		(22) 500 g Hafer 250 g Heu	10,7	5,5	1500	825	1015	[illegible]	0,477	9,558	1,135	1 : 6,7							
12.		dto.	10,7	5,5	1000	560	1017	[illegible]		6,914	0,931	1 : 7,4	2,940	18,569	1,964	12,387	— 9,75	+ 1,43	
13.	35,3 kg	dto.	10,7	5,5	1000	315	1025	[illegible]		6,780	0,319	1 : 21,2							
		(23) Summa	32,1	16,6	3500	1700		[illegible]		23,282	2,385			18,569		12,387	— 9,75	+ 1,43	
		(24) tägl. Durchschnitt	10,7	5,5	1170	570	1019	[illegible]		7,760	0,795		2,940	6,19	1,964	4,129	— 3,25	+ 0,48	

1. Date; 2. Weight; 3. Uptake; 4. Feed; 5. Contained therein; 6. Drinking water; 7. Urine; 8. Quantity; 9. Specific weight; 10. Oats; 11. Hay; 12. Daily Average; 13. Fasting period; 14. 400 g oats, 150 g cottonseed flour, 75 g lactose; 15. 10 g oats, 5 g cottonseed flour, 37 g lactose; 16. 46 hay, 79 cottonseed flour, 75 g subcutaneous sugar; 17. 100 g oats, 50 g sugar, 100 g cottonseed flour; 18. 100 g oats, 200 g cottonseed flour, 150 g sugar; 19. 500 g oats, 250 g hay; 20. Total; 21. Daily average; 22. Daily average; 22. 500 g oats, 250 g hay; 23. Total; 24. Daily average; 25. Excretions; 26. Feces; 27. Urine; 28. N %; 29. Feces; 30. Balance; 31. Remarks;
32 Uniform pre-feeding from 15 September - 19 September. Feces from period 19 - 23 September were taken as an average sample for analysis;
33. Urine gave a clear reaction with indicators throughout the entire starvation period, though this decreased towards the end of the period; no albumen and no reduction;
34. Injection of 5 cc toxin;
35. The animal was given subcutaneously 75 g lactose dissolved in 750 water at 6½ N.p.m.
[not identified]; unwägbar = unweighable; summa = total.

Nitrogen was determined according to Kjeldahl, phosphoric acid was analyzed by weight, since customary titration proved unsuitable for urine. Control determinations were done in all cases, and, in cases where the results appeared striking, 3-4 analyses were done. For P_2O_5 determination substances were burned in Kjeldahl flasks according to the method of Naumann. Due caution was exercised in the molybdenum and magnesium precipitation and calcination of $Mg_2P_2O_7$ (Zschr. f. analyt. Chem., Vol 33, p 362). Weight differences between individual determinations were rarely more than 0.0005 to 0.04 g $Mg_2P_2O_7$[1]).

Ammonia determination was done according to Schlosing with 100 cm^3 urine and 25 cm^3 1/5 N H_2SO_4. The jars were always left with the slaked lime at even temperatures for 4 x 24 hours. The difference was at most 0.1 cm^3 1/10 N NaOH.

Analysis of Feed

The fodder was examined in average samples from each purchase which, preferably, was sufficient for one experiment. The fodder was preserved at temperatures as uniform as possible and in uniform humidity.

Hay (airdried)	samples of	8 Jan 1900	23 Feb 1900	23 May 1900	14 Sep 1900
Raw protein		8.9 %	9.5%	11.4 %	9.0 %
Pure protein		7.7 %			
Fat		2.0 %			
Raw fibres		33.6 %			
N-free substances		44.1 %			
Ash		6.8 %			
Water		4.6 %			
P_2O_5				0.394%	0.385%

Oats (airdried)	samples of	26 May 1900	14 Sept 1900
Raw protein		12.2 %	11 %
Pure protein		11.3 %	
P_2O_5		0.955%	1.000%

1) In several cases 100-200 cm^3 of urine made up to 1 liter was only so slightly turbid after addition of the Mg mixture that in due consideration of possible errors the precipitate was considered below what could be weighed. Compare Exp. 3 15 Sept, Exp. 4, 30 Jan to 31 Jan 1901. Control determinations of phosphoric acid were done in experiment No 4 on 15, 18, 22, 24, 26, 28, 30 and 31 Jan and 4 Feb, further also in the mixed urine of 16-20, 21-25, 26-28 Jan and 29 Jan-4 Feb.

Rye bran (airdried) samples of 8 Jan 1900

Raw protein	17.9 %
Pure protein	12.6 %
Fat	3.3 %
Raw fibres	4.7 %
N-free substances	58.7 %
Ash	5.0 %
Water	10.3 %

Cottonseed flour

Raw protein	43.7 %
Raw fat	14.7 %
P_2O_5	2.577 %

Finally I wish to thank Prof. L. Krehl who has encouraged me to undertake this investigation and lent me his support during its progress, also Prof. Dr. E. Romberg for his kindness in placing the facilities of the Medical Out-patient Clinic at my disposal.