Gehäuse pyramidenförmig, glatt, aber nicht glänzend; fein sandig. Weiter ganz nach CUSHMANs Beschreibung. Meine Exemplare sind aber viel kleiner als die von CUSHMAN, (bis zu 2.50 × 1.50), 0.33 × 0.22 mm. — Oligozän von Niekerk.

Cristellaria depauperata Rss. var. *costata* Rss. (Fig. 4, 4*a*).

A. REUSS. Denkschr. K. Ak. d. Wiss. Wien, 25, 1866, S. 30—31, T. IV, Fig. 5, 6.

Das Gehäuse seitlich zusammengepresst, etwas länger als breit, der letzte Umgang aus 5—6 mehr oder wenig gewölbten Kammern gebildet, die durch erhöhte Suturrippen voneinander getrennt sind. Suturen etwas hinterwärts gebogen, Kammern etwas dreieckig. Die Mündung ist eine kleine Spalte an der Oberseite der letzten Kammer. Die Septalfläche der letzten Kammer ist von rippenartigen Leisten begrenzt. Oberfläche glatt; Länge: 1.81—2 mm, Breite: 1.47—1.6 mm, Dicke: 0.61 mm. — Oligozän von Niekerk.

Rotaliatina bulimoides (Rss) (Fig. 5).

Rotalina bulimoides Rss. Zeitschr. D. Geol. Ges. 3, 1851, S. 77, T. V. Fig. 38.
Rotaliatina Cushman. Contr. Cushm. Lab. For. Res. vol. I, pt. 1, 1925, S. 4.

Das glatte Gehäuse bildet eine hohe, aus 3 Umgängen bestehende Spira, oben breit und unten zugespitzt, sehr eng genabelt. Der letzte Umgang zählt 6—7 Kammern, die durch kaum sichtbare Nähte getrennt sind. Die Mündung ist eine längliche, schmale Spalte am inneren Rande der letzten Kammer. Höhe 0.37 mm. — Miozän von Almelo (54—56 m).

Cristellaria subaculeata Cushm. var. *glabrata* Cushm. (Fig. 6, 6*a*).

J. A. CUSHMAN. U.S.N.M. Bull. 104 (4), S. 124, T. XXXIII, Fig. 2—3; T. XXXIV, Fig. 3.

Das Gehäuse länger als breit, abweichend von *Cr. subaculeata* C. durch einen längeren nicht-spiraligen Teil und viel stärkere Ausstattung mit Knoten und Stacheln. Die Zahl der Kammern des spiraligen Teiles ist nicht mehr ersichtlich; in dem geraden Teile 3 bis 4. Zwischen den Suturen kann die Wand bisweilen fast glatt sein. Masse: 0.9 × 0.5 mm. — Miozän von Delden (96—103 m).

Cristellaria vaginalis Rss. (Fig. 7, 7*a*).

A. REUSS. Sitz. Ber. K. Ak. d. Wiss. Wien. 48, 1865, S. 50—51, T. IV, Fig. 49.

Das Gehäuse kurz und ziemlich breit, scheidenförmig. Der untere Teil zu einer kleinen Spira eingerollt, die letzten 4 Kammern in gerader Reihe übereinander stehend. Der Rücken abgerundet winklig, die Bauchseite des geraden Teiles breiter. Die letzte Kammer stark aufgeblasen. Die erste sichtbare Kammer ist sehr klein. Alle Kammern sind breiter als hoch. Die letzte Kammer ist doppelt so hoch wie die vorletzte. Nähte kaum eingesenkt, nahezu linear. Die Mündung dorsal, gestrahlt. Masse: 1.24 × 0.54 mm. — Oligozän von Vennewieck.

Guttulina cf. *cliffdenensis* Parr. u. Coll. (Fig. 8, 8*a*, 8*b*).

PARR und COLLINS, Proc. Roy. Soc. of Vict. new ser. 50, pt. I, S. 196, T. XV, Fig. 7*a*—*c*.

Gehäuse glatt, ziemlich flach, oval, an der Unterseite ein wenig zugespitzt, was bei *Gutt. Cliffordensis* Parr. u. Coll. nicht der Fall ist. Kammern nach der Länge gezogen, jedenfalls länger als breit; Suturen deutlich, ein wenig eingesenkt, was bei *Gutt. cliffordensis* Parr. u. Coll. nicht der Fall ist. Anderseits zeigen unsere Exemplare einige Uebereinstimmung mit *Gutt. insignis* Rss. (Sitz. Ber. K. Ak. Wiss. Wien, 50, 1864, S. 470, T. IV, Fig. 4), aber diese ist fast doppelt so gross wie unsere Exemplare und hat mehr sichtbare Kammern. Diese Form wird von CUSHMAN und OZAWA gleichgestellt mit *Sigmomorphina crassa* (Roemer) (Proc. U.S.N.M. 77, Mon. Polymorphinidae). Hier sind die Suturen wie bei unseren Exemplaren auch ein wenig eingesenkt. Die Masse sind aber grösser als bei unseren Exemplaren, (Länge: 1.20 mm, Breite: 0.94 mm, Dicke: 0.45 mm). Die Exemplare von PARR und COLLINS sind aus dem Ober-Oligozän von Neu-Seeland, die von REUSS aus dem Septarienton Nord-Deutschlands, unsere aus dem Ober-Mittel-Miozän von Rekken.

Physiology. — *Studies on phosphorus metabolism in normal and rachitic rats with a radioactive phosphorus isotope.* II. *The total phosphorus and lipin phosphorus content and the formation of lipin phosphorus.* By M. J. L. DOLS and B. C. P. JANSEN (Laboratory of Physiological Chemistry, University, Amsterdam) and G. J. SIZOO and F. BARENDREGT (Natuurkundig Laboratorium, Vrije Universiteit, Amsterdam.) (Communicated by Prof. G. VAN RIJNBERK.)

(Communicated at the meeting of October 29, 1938.)

Introduction.

In a previous paper [1]) the results were communicated of some experiments on phosphorus metabolism in normal, rachitic and rachitic rats treated with vitamin D, by which the distribution, absorption and re-excretion in relation to the deposition in bone of the active phosphorus administered were examined.

It was found, that the distribution of the phosphorus administered could be followed up easily in the organism; almost the whole of the quantity of the labelled phosphorus administered could be recovered. A very rapid entrance of the labelled phosphorus into the bone was perceptible in all cases. Furthermore it was observed that one hour after the injection into the tail vein, a considerable amount of the radioactive phosphorus was re-excreted into the small intestine, whereas the phosphorus within one hour had disappeared entirely from the blood in several rats. So far as the gross absorption was concerned no difference could be observed in the normal and rachitic rats, whereas the same can be said about the re-excretion into the gut.

In this paper we wish to discuss now the formation of lipin phosphorus after injection of inorganic phosphorus and also the Ca content, the total P content and the lipin P content in normal and rachitic rats.

Procedure and methods.

The rats used were of the albino type; they were weaned on the 21st day and then used in our experiments. Most of them then had a weight of 35—40 grams. Every rat was kept in a zinc cage with a crossbarred floor and placed in a room from which all daylight was excluded. The

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[1]) Proc. Kon. Akad. v. Wetensch., Amsterdam, **40**, 547 (1937).

rats were divided into two groups in such a manner, that the rats in the comparable groups are from the same litters. Both groups were fed for about three weeks the STEENBOCK–BLACK diet. Besides, the check group received three times a week a sufficient dose of halibut oil, whereas the other rats only got a solution of carotene in peanut oil. After three weeks the rats were x-rayed to check the diets and thereupon used in the experiments.

The radioactive phosphorus used was obtained for a part by neutron bombardment of sulphur with a radium-beryllium mixture (100 mg. Ra) [1]), whereas moreover we got a generous gift of Prof. LAWRENCE from the University of California. This radioactive phosphorus was prepared by the bombardment of red phosphorus by high speed deuterium ions generated in a cyclotron.

The labelled phosphorus was administered as sodiumphosphate in an aqueous solution of pH 6—7. For that purpose a fixed quantity of the radioactive solution was injected into the tail vein. A certain time after the injection, in these experiments after ½, 1, 3 and about 20 hours, the rats were decapitated, whereas the blood was caught in a tarred cup or on an ashless filter. The body, the head and the filter with blood were immediately plunged into liquid air, then crushed in a tissue crusher [2]), in which the material as well as the whole crusher could be kept cold with carbon dioxyde snow and further pulverized in a cooled laboratory mill. Finally, the pulverized rat was kept in a frozen condition in solid carbon dioxyde until the preparations to be measured were made and the chemical estimations were completed. In this manner, no post mortem changes in the rat tissues during the process of its preparation for analysis or for the determination of the radioactivity are expected to occur and it is very easy to make reliable bulk samples of the whole rat.

In the samples of the pulverized rat the Ca, the total P, the lipin P and the remaining P were estimated.

For the analysis of the calcium and total phosphorus and for the lipin phosphorus, big samples of the pulverized rats were used, viz. 20 grams for each estimation, i.e. about a third of the whole rat. The sample of the pulverized rat, which had to be used for the estimation of the calcium and the total phosphorus was carbonized in an oven at 200 degrees centigrade. Both for the estimation of the calcium and total phosphorus 2 grams of the charred mass were then ashed according to NEUMANN, after which the estimations were done. The phosphorus was estimated using the colorimetric method of FISKE and SUBBAROW, whereas the calcium was estimated by precipitation as oxalate and titration with permanganate.

The sample of the pulverized rat, employed for the estimation of the

[2]) J. B. GRAESER, J. E. GINSBERG and TH. E. FRIEDEMANN, J. biol. Chem., **104**, 149 (1934).

lipin phosphorus, was extracted with a mixture of absolute alcohol-ether 3 : 1; then a fourth of the extract was evaporated to dryness, ashed according to NEUMANN and the phosphorus again estimated colorimetrically.

The radioactivity was measured in at most four preparations of each animal, namely, the blood, the total P fraction, the lipin P fraction and the remaining P fraction.

The preparations to be measured were made as has been described previously. Therefore, the dried blood and the P fractions were suspended in ether, whereafter the suspension was poured out into a flatly grinded ring of glas, which was placed on a tarred aluminium foil. Then the ether was evaporated at room temperature after which the preparation could be measured.

The determination of the radioactive phosphorus isotope in these preparations was done with the compensation ionization chamber, whereby the ionization current produced by the radioactive phosphorus was compared each time with the ionization of a constant source [3]).

All the activities were reduced to the moment at which the activity of the solution administered was measured.

Finally, it is very important to draw attention to the fact, that we have also examined the adequacy for our method for extraction of the phospholipins. Therefore, both a quantity of 500 and 1500 radioactive units were added to 30 grams fresh rat tissue. After the tissue had been frozen and pulverized, the lipin phosphorus was extracted as mentioned before with a mixture of absolute alcohol-ether 3 : 1. After the extract was evaporated to dryness, the half of the residue was extracted again with carefully dried ether. After this extraction the residue was ready to make the preparations which had to be measured.

The second part of the dried residue was treated as has been described by HAHN and HEVESY [4]). It was also extracted with carefully dried ether, evaporated again to dryness and dissolved a second time in ether in the presence of a large excess of finely powdered unlabelled sodium phosphate. By shaking this solution and repeating this treatment it is possible to get rid of the slightest trace of inorganic labelled phosphorus. Therefore, we are glad to confirm the observation of HAHN and HEVESY. In our opinion, however, the treatment according to HAHN and HEVESY, which takes up much time, is not necessary in our experiments, as the highest activity measured in the extract treated as usual was 0.4 units, i.e. 0.025 % of the active phosphorus administered or 0.002 % of the injected phosphorus in 1 mg. lipin phosphorus. As is clearly shown in table II, this value has no influence on the results obtained in our experiments. In our investigations the exactness of the first mentioned method was sufficient.

[3]) G. J. SIZOO and C. P. KOENE, Physica, **3**, 1053 (1936).
[4]) L. HAHN and G. HEVESY, Skand. Arch. Physiol., **77**, 148 (1937).

Discussion of the experiments.

In these experiments 90 rats in all were used; in 42 of them the calcium content of the whole body was estimated, whereas in 66 of these rats the total phosphorus content was estimated too. The lipin phosphorus was estimated in all the 90 rats, whereas 72 of these rats were used for the investigation on the formation of lipin phosphorus from the inorganic phosphorus administered.

In rickets, as is generally known, the calcium and the total phosphorus of the bones have diminished. As the skeleton contains 98 % of all the calcium in the whole body, whereas the phosphorus content in the skeleton is about 80 % of the total phosphorus in the whole animal, the diminution of the values for calcium and total phosphorus may be expected.

In a previous paper [5]) it was shown, that only in cases of severe rickets the difference between the means of total P in normal and rachitic rats does seem to be significant. In the cases of very slight rickets no difference between the means exists, whereas in the cases of moderate rickets the significance of the difference between the means is not at all convincing.

The rachitic rats in these experiments, used for the estimation of the total phosphorus generally had a severe rickets; only in a few cases moderate rickets was observed. The results of these estimations, which are tabulated in table I, show, that the total phosphorus content of the rachitic rats has significantly decreased. The same thing can be said of the calcium content of the whole body; in rachitic rats the calcium content had sharply diminished. As was told in a previous paper [5]), as far as we know, figures with regard to the lipin phosphorus of the whole animal have never been published before. On the other hand, there is a lot of investigators, who have estimated the phospholipins in several organs from normal and rachitic animals [6]).

The results of our estimations on the lipin phosphorus content of 90 normal and rachitic rats are also tabulated in table I, in which the figures are reduced into percents in the dried matter.

It was found after statistical treatment of the figures for the lipin phosphorus content in the dried matter from normal and rachitic rats, that the slight increase in the rachitic rats was not significant. Nevertheless, it is a very interesting observation, that in opposition to the total phosphorus, the lipin phosphorus in rachitic rats has not diminished. On the contrary, the lipin phosphorus content reduced into percents of the total phosphorus has clearly increased in the rachitic rats. The lipin phosphorus content in normal rats is 10.82 % ± 0.3 % of the total P, whereas in rachitic rats it is 12.33 % ± 0.3 %. The difference here amounts to 1.51 ± 0.42 % and is strikingly significant.

A second question which is discussed in this paper acts with the formation of lipin phosphorus after injection of inorganic phosphorus. In earlier papers the formation of lipin phosphorus was studied by ARTOM and his collaborators [8]), who showed, that about 9 hours after the ingestion of fat and radioactive phosphorus, comparatively large quantities of the active phosphorus were already found in the phospholipins of liver and gut of a rat.

HAHN and HEVESY [9]) showed that, in one hour after the subcutaneous injection of labelled phosphate, labelled lecithin was already formed in the brain tissue of a fully grown rat. In a provisional paper [10]) we were able to establish, that both in normal and rachitic rats, within half an hour after the injection of the active phosphorus a considerable quantity was present in the phospholipins of the whole animals. In prolonged researches with 72 rats the formation of lipin phosphorus after injection of inorganic phosphorus was investigated. As was mentioned before, the rats were decapitated ½, 1, 3 and 20 hours after the injection. The radioactivity was measured in at most four preparations of each animal.

The blood figures confirmed our previous results [11]); though the divergency in the individual results was very large, it was found, that already within half an hour, both in the normal and rachitic rats, the active phosphorus for the greater part had disappeared from the blood. The results of the measurings of the radioactivity of the total phosphorus fraction were also fully in accordance with our earlier observations [11]). The reduction of the measured radioactivity of this fraction on the total phosphorus content of the whole body showed, that almost the whole of the quantity of the labelled phosphorus administered could be recovered.

A very important and unexpected observation was made on the formation of lipin phosphorus in normal and rachitic rats. The results of these estimations are tabulated in table II. It was found here, that the

TABLE I.

Group	Number of Rats	Calcium % dried matter	Number of Rats	Total P % dried matter	Number of Rats	Lipin P % dried matter
Normal	21	2.72 ± 0.13	33	1.82 ± 0.04	45	0.197 ± 0.004
Rachitic	21	2.08 ± 0.08	33	1.63 ± 0.03	45	0.201 ± 0.003
Difference		0.64 ± 0.15		0.19 ± 0.05		0.004 ± 0.005
t (FISHER)		4.3		3.8		0.8
P (FISHER) [7])		0.00		0.00		

[5]) M. J. L. DOLS, Acta brevia Neerlandica, **8**, 117 (1938).
[6]) R. NICOLAYSEN, Biochem. J., **30**, 1329 (1936).
[7]) R. A. FISHER, Statistical Methods for Research Workers, 4th Ed. 1932.
[8]) C. ARTOM et al., Arch. Intern. Physiol., **45**, 32 (1937).
[9]) L. HAHN and G. HEVESY, Skand. Arch. Physiol., **77**, 148 (1937).
[10]) Nature, **141**, 77 (1938).
[11]) Nature, **139**, 1068 (1937).

percentage of the active phosphorus present in 1 mg. of the lipin phosphorus formed in the first half hour after the injection of active sodium phosphate seems to be larger in the normal than in the rachitic rats. The statistical treatment of these figures, however, showed that the difference in favour of the normal rats was not significant.

TABLE II.

Group	Number of Rats	Interval after injection	Percentage of injected P in 1 mg. lipin P	Difference in favour (+), at the cost (—) of the normal rats	t (FISHER)	P (FISHER)
		h. m.				
Normal	13	0 30	0.381 ± 0.057	+ 0.061 ± 0.09	0.68	
Rachitic	17	0 30	0.320 ± 0.070			
Normal	8	1	0.212 ± 0.038	— 0.096 ± 0.052	1.85	0.08
Rachitic	8	1	0.308 ± 0.036			
Normal	5	3	0.520 ± 0.059	— 0.600 ± 0.113	5.31	0.00
Rachitic	5	3	1.120 ± 0.096			
Normal	8	20	1.628 ± 0.163	— 0.904 ± 0.222	4.08	0.00
Rachitic	8	20	2.532 ± 0.150			

The results of the estimation of the radioactivity in the lipin phosphorus fraction of animals decapitated one hour after the injection of the active sodium phosphate, however, were quite different from what we saw just now. It was clearly shown from the figures that the percentage of the active phosphorus in 1 mg. lipin phosphorus from rats decapitated one hour after the injection of the active phosphorus was larger in the rachitic than in the normal rats. The same results were obtained in animals decapitated 3 and 20 hours after the injection of the active phosphorus. The statistical treatment of the figures confirmed the conclusion, that the difference of the percentage of the injected phosphorus present in 1 mg. lipin phosphorus was very significant, in favour of the rachitic rats.

From these results, it seems that an increased formation or a decreased destruction of the lipin phosphorus takes place in rachitic rats. We do not launch into speculations, the fact, however, that in rachitic children and animals the phosphatase content of the blood serum has increased, as was shown by several investigators [12]), does not point to a decreased destruction of lipin phosphorus in rachitic rats, but in preference to an increased formation.

The observation, that the absolute value of the injected phosphorus

[12]) J. SMITH, Arch. Dis. Child., **8**, 215 (1933); A. BODANSKY and H. L. JAFFE, Amer. J. Dis. Child., **48**, 1268 (1934); R. H. COMMON, J. Agric. Sci., **26**, 492 (1936).

present in 1 mg. lipin phosphorus in rats decapitated one hour after the injection was smaller than in the rats decapitated half an hour after the injection of active sodium phosphate, probably must be explained in this way, that in this experiment the sodium phosphate was injected intraperitoneally, whereas in the other experiments it was done intraveneously.

In prolonged investigations we now have started to investigate the influence of vitamin D on the formation and destruction of lipin phosphorus in rachitic rats treated with vitamin D. Especially the suggestion of MCGOWAN [13]), that vitamin D acts by the setting free of phosphoric acid from the lipins will be examined. The mentioned result, that the lipin phosphorus content in percents of the total phosphorus has clearly increased in rachitic rats, speaks for his opinion.

Summary and conclusions.

In this paper the experiments are reported on the formation of lipin phosphorus after injection of radioactive sodium phosphate in normal and rachitic rats. Besides, the results of the estimation of the Ca content, the total P content and the lipin P content in the whole rat are published.

As was clearly shown, the total phosphorus content of the rachitic rats has significantly decreased. The same thing can be said of the calcium content of the whole rat; in the rachitic rat it has sharply diminished. With regard to the lipin phosphorus content a very important observation has been done. It was found that, in opposition to the total phosphorus, there was no decrease in the lipin phosphorus content of the rachitic rats calculated on the dried matter. The lipin phosphorus, however, reduced into percents of the total phosphorus content has clearly increased in the rachitic rats.

The results on the formation of lipin phosphorus in normal and rachitic rats were also very important. It was clearly established, that in the first half hour after the injection no difference exists between the normal and rachitic rats. In rats decapitated one hour, three hours or twenty hours after the injection of the active phosphorus, a significant difference in the quantity of injected phosphorus present in 1 mg. of the lipin phosphorus exists in favour of the rachitic rats. Therefore, it seems that an increased formation or a decreased destruction of lipin phosphorus takes place in the rachitic rats.

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[13]) J. P. MCGOWAN, Biochem. J., **27**, 943 (1933).