CXXI. SOME CONSIDERATIONS REGARDING AND INVESTIGATIONS INTO CALCIUM AND PHOSPHORUS METABOLISM.

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During an investigation into the nature of vitamin D action, the results of which are recorded elsewhere [McGowan *et al.*, 1931; McGowan, 1933], certain phenomena were observed with regard to Ca and P metabolism which were deemed worthy of further inquiry; a perusal of the literature supplied others, and an attempt has been made in this investigation to answer some of the questions arising in this way.

It is desirable, for clarity, first to discuss briefly certain factors which play important parts in the matter under investigation.

Gastric acidity. If one assumes that calcium is absorbed either as organic calcium salts occurring in the food or as calcium chloride formed by the interaction of calcium salts, such as the carbonate of the food, with the gastric hydrochloric acid, whilst phosphoric acid is absorbed in the form of its salts with alkali metals or of acid salts of the alkaline earths, it is evident that the gastric acidity must exercise an important influence over the absorption of Ca and P.

So long as the amount of the gastric hydrochloric acid normally excreted is in excess of the total uncombined base and carbonates of the food, conditions will be favourable for solubility, and therefore complete absorption, of Ca and P. If on the other hand, the gastric juice is insufficient to neutralise all the calcium (and alkali) carbonates of the food, whilst a moderate proportion of the calcium may be converted into calcium chloride and absorbed, the phosphates will be precipitated as tricalcium phosphate to an extent depending on the excess of calcium carbonate and to this extent will escape absorption.

It appears, therefore, that, contrary to Stewart and Percival [1928] and others, a deficient absorption of Ca can occur only in response to absolute deficiency of Ca in the diet (so far at least as dietary composition alone is concerned) whilst excess of Ca in the diet can prevent absorption of an otherwise adequate amount of P. Optimum conditions for absorption and subsequent utilisation of Ca and P will be obtained when these are supplied in the diet in moderate and approximately equivalent amounts, with the proviso that the total uncombined base of the food must be considerably less than sufficient to neutralise the gastric hydrochloric acid[1]. As regards utilisation, if Ca or P is rapidly absorbed in large quantity into the blood, utilisation will be affected in that, the body being unable to "fix" such large amounts, these will be immediately excreted in order to keep the composition of the blood normal.

[1] The bearing of the condition "achlorhydria" on the present subject will be discussed in a subsequent communication.

Intestinal excretion of Ca and P. In spite of the lack of quantitative data on normal animals, it has been firmly established [*cf.* Mendel and Benedict, 1909–10; Walsh and Ivy, 1927–28; Stewart and Percival, 1928] that Ca and P are actually excreted by the intestine: in dogs the amount of Ca excreted in this way is said to be independent of that taken in the diet [Taylor and Weld, 1932], whilst in herbivora the intestinal excretion of tricalcium phosphate forms an important mechanism for regulating acid-base equilibrium.

Ca and P in the blood. It is not clear that calcium phosphate really exists in supersaturated solution in the blood as has been claimed [Holt, La Mer and Chown, 1925; Peters and Van Slyke, 1931]: the failure of calcium phosphate to precipitate from blood-serum in spite of the fact observed by Adler [1919], that precipitation of calcium phosphate occurs from an artificial "inorganic" blood-serum, is probably rather to be explained by the effect of the serum-proteins in diminishing ionisation [Shear and Offner, 1931; *cf.* also Pauli and Schon, 1924]. The action seems to be one of preventing the formation of the insoluble calcium phosphate rather than of keeping it in solution after it has been formed. Whatever the nature of the mechanism may be, however, which normally prevents precipitation of calcium phosphate from blood-serum, owing to the solubility properties of this salt, it is evident that any influence, such as increase in Ca or P concentration which would tend to bring about its precipitation, will be more effective at an alkaline reaction.

In point of fact under ordinary conditions the normal alkaline state of the blood is usually associated with access of considerable quantities of calcium to the blood and hence with the usually found high Ca : low P relationship (the reverse ratio is produced experimentally when blood-Ca is lowered by injection of alkaline phosphates [Binger, 1917]). On the other hand, in conditions of acidity of the blood such as is produced by the administration of calcium chloride, there is an increase both in the Ca and P concentrations [Salvesen *et al.*, 1924; Collip, 1926, 1, 2], a state of affairs which also occurs in the terminal stages of hyperparathyroidism and which may be brought about artificially by the simultaneous injection of $CaCl_2$ and acid sodium phosphate [Collip, 1926, 1]. Thus in acid states of the blood there is no fixed ratio between the Ca and P concentrations.

The claim of Howland and Kramer [1921] that the maintenance of the Ca × P product of the blood above a certain level is to be regarded as diagnostic of freedom from rickets may be considered in this connection. The ascription of any significance to this product[1] is open to theoretical objection since no account is taken of the state of combination and active concentrations of the elements under consideration: moreover, the results of other workers [Greenwald, 1931; Farquharson and Tibbets, 1931; Stearns and Knowlton, 1931; Dupré and Semeonoff, 1931; Bourne and Campbell, 1932; Theiler and Green, 1932; present investigation] show that the Ca × P product cannot in fact be regarded as in any sense an index of normality, as has been suggested by Howland and Kramer [1923]. No attempt has been made to explain the frequent abnormal increases in the Ca × P product which may be observed, and the view that the product defines the physiological state of the organism in any way becomes manifestly absurd when we consider that identical values may be found for it in normal and abnormal animals with the ordinary high Ca and low P ratio and in the tetanic animal with low Ca and high P.

Such "reciprocal" relationship as may exist between the Ca and P of the

[1] Considerable confusion has crept into this subject by forgetting, in reading blood-Ca and -P concentration charts, that Ca × P involves a geometric not an arithmetic conception.

blood appears rather to be an expression of a balanced action between calcium and phosphorus compounds in the blood, the point of equilibrium of this action being determined by the effect of the reaction of the blood on the solubility of the least soluble of these compounds (calcium phosphate) which it contains.

In all work involving determinations of Ca and P in blood it is necessary to remember that the concentrations of these elements actually observed are the resultants of a large number of different processes such as absorption, excretion, endogenous formation and fixation, and without consideration of these factors, particularly that of diet [*cf.* Bourne and Campbell, 1932; Dupré and Semeonoff, 1931] the figures may have little or no significance.

Experimental observations.

A series of CaO and P_2O_5 balance experiments was carried out to test the effect of the amounts of CaO and P_2O_5 and their ratio in the food on their retention and excretion.

Full-grown rabbits, weighing 2000 to 2300 g. were employed, the experiments being carried out in the usual way with the usual precautions. The stock ration consisted of equal parts of bran, sharps and "Porage" oats, to 24 lbs. of which was added 1 lb. of fish meal. For roughage, 100 g. turnip were given each rabbit daily and no account was taken of this in the balance. Water was freely available.

The composition of the various rations fed are given in Table I.

Table I.

No.	Description	CaO %	P_2O_5 %	Ratio CaO/P_2O_5
1	Stock ration with high $CaCO_3$ content	4·5589	1·6193	2·8
2	,, "balanced" with $CaCO_3$	1·1512	1·8476	0·62
3	,, + steamed bone flour ($Ca_3(PO_4)_2$)	4·5325	4·7772	0·95
4	,, + dicalcium phosphate	3·0829	5·0303	0·60
5	Stock ration	0·4200	1·7883	0·23
6	,, *minus* fish meal	0·0970	1·4848	0·065
7	,, + medium Na_2HPO_4	0·0432	3·2204	0·013
8	,, + high Na_2HPO_4	0·0130	4·4695	0·0029

It will be seen that the ratio CaO/P_2O_5 diminishes considerably from ration 1 to ration 8 in the table. As the animals exercised some degree of selection from the ration, the ratios given here for the food offered differ slightly from those of the food actually eaten (Table II).

Except where otherwise stated, the rabbits were fed on the experimental ration for a week before collection of urine and faeces for analytical purposes was commenced. The results of the various experiments will be discussed together and are collected in synoptic form for this purpose, in Table II, in six groups: the figures are average daily ones.

Column A represents the CaO/P_2O_5 ratio in the food eaten in the various experiments. This differs slightly, owing to selection, from that in Table I but diminishes in the same way from group I through group VI to group V.

Column O shows that the amount of P_2O_5 consumed in the various experiments is high and, with the exception of the steamed bone flour and dicalcium phosphate experiments, roughly equal. The diminishing CaO/P_2O_5 ratio series is due therefore to diminishing calcium intake as is shown in column G.

The CaO balance follows the same diminishing order except in the case of rabbit *C*, on steamed bone flour, where a high negative balance is in all

Table II.

Rabbit	Dates	Days	P_2O_5 Intake nett g.	P_2O_5 Urine g.	P_2O_5 %	P_2O_5 Faeces g.	P_2O_5 %	P_2O_5 Balance g.	CaO Intake g.	CaO Urine g.	CaO %	CaO Faeces g.	CaO %	CaO Balance g.	Food eaten ratio CaO to P_2O_5	Fraction intake P_2O_5 excreted in urine	Group
	Q	P	O	N	M	K	I	H	G	F	E	D	C	B	A	X	
Stock ration + high $CaCO_3$:																	
A	8–12. vii. 31	5	0·8089	0·0472	7·15	0·6128	92·85	+0·1480	1·6159	0·7976	63·2	0·4645	36·8	+0·3738	1·99	0·05	I
B	7–12. vii. 31	6	0·9684	0·0088	1·3	0·6584	98·7	+0·3012	2·2889	0·4040	35·8	0·7254	64·2	+1·1595	2·3	0·009	
C	25–30. vii. 31	6	0·9354	0·0123	1·4	0·8831	98·6	+0·0400	2·1233	0·6572	45·1	0·7989	54·9	+0·6672	2·2	0·01	
C^1	13–26. x. 31	14	0·8535	0·0398	4·5	0·8347	95·5	−0·0210	1·9434	0·5421	44·5	0·6752	55·5	+0·7261	2·2	0·04	
H	29. xi.–11. xii. 31	13	1·0883	0·0346	2·8	1·1810	97·2	−0·1273	2·3557	0·5679	36·1	1·0069	63·9	+0·7809	2·08	0·03	
H^1	18–21. xii. 31	4	1·2898	0·0567	5·7	0·9414	94·3	+0·2917	2·8036	0·9338	52·8	0·8348	47·2	+1·0350	2·17	0·04	
Stock ration "balanced" with $CaCO_3$:																	
E	28. x.–9. xi. 31	13	1·3622	0·2359	18·9	1·0092	81·1	+0·1171	0·7034	0·0274	4·8	0·5458	95·2	+0·1301	0·49	0·17	II
Stock ration + steamed bone flour:																	
C	2–5. viii. 31	4	0·8657	0·2282	20·8	0·8668	79·2	−0·2293	0·3214	0·1479	18·8	0·6396	81·2	−0·4661	0·37	0·26	VI
G	2–11. xii. 31	10	1·9882	0·2857	13·4	1·8450	86·6	−0·1424	1·3718	0·1376	10·8	1·1390	89·2	+0·0952	0·69	0·14	
Stock ration + dicalcium phosphate:																	
G	12–23. xii. 31	12	1·8500	0·4909	22·9	1·6566	77·1	−0·2975	0·9416	0·0415	4·3	0·9128	95·7	−0·0127	0·50	0·26	
Stock ration:																	
C	17–22. vii. 31	6	1·2075	0·4881	49·9	0·4902	50·1	+0·2292	0·2148	0·0390	16·2	0·2033	83·8	−0·0275	0·17	0·40	III
C^1	30. ix.–12. x. 31	13	0·8368	0·3767	47·5	0·4160	52·5	+0·0441	0·1660	0·0115	6·6	0·1620	93·4	−0·0075	0·19	0·45	
D	30. ix.–12. x. 31	13	1·2849	0·2212	24·9	0·6656	75·1	+0·3981	0·2280	0·0041	1·2	0·3512	98·8	−0·1273	0·17	0·18	
G	17–27. xi. 31	11	1·3051	0·4264	40·1	0·6370	59·9	+0·2417	0·1478	0·0110	5·1	0·2039	94·9	−0·0671	0·11	0·32	
Stock ration less fish meal:																	
D	13–21. x. 31	9	1·0856	0·2626	22·1	0·9240	77·9	−0·1010	0·0694	0·0018	0·6	0·2942	99·4	−0·2266	0·07	0·24	IV
G	10–21. i. 32	12	0·9825	0·2893	33·6	0·5699	66·4	+0·1233	0·0391	0·0026	1·4	0·1848	98·6	−0·1483	0·04	0·29	
H	10–21. i. 32	12	1·3681	0·4989	38·0	0·8125	62·0	+0·0567	0·0564	0·0024	1·1	0·2234	98·9	−0·1694	0·04	0·36	
Stock ration + medium Na_2HPO_4:																	
G	22. i.–2. ii. 32	12	0·7973	0·2718	38·1	0·4422	61·9	+0·0833	0·0298	0·0024	1·8	0·1299	98·2	−0·1025	0·037	0·33	
Stock ration + high Na_2HPO_4:																	
G	3–9. ii. 32	7	0·8682	0·2338	52·7	0·2097	47·3	+0·4247	0·0015	0·0014	1·8	0·0766	98·2	−0·0765	0·0018	0·27	V

probability due to the animal having been on a high $CaCO_3$ diet immediately previously (Ca fixed lightly as $CaCO_3$).

The percentage excretion of CaO in the urine—column E—has the same trend; the percentage excretion of CaO in the faeces—column C—is the inverse of this. The slight irregularity in the lower figures of these series is probably due to difficulty of accurate estimation of the very small quantities of CaO involved.

Particular attention is directed to the very high positive CaO balance in the high $CaCO_3$ experiments, which is coupled with a low P_2O_5 balance and a high percentage excretion of P_2O_5 in the faeces. This indicates two things, first, that CaO can be fixed in large quantities in the body without the aid of P_2O_5, and second, that a large quantity of P_2O_5 is rendered insoluble in the intestine by $CaCO_3$ and hence is not absorbed. The alternative to this latter conclusion, namely that the insoluble $Ca_3(PO_4)_2$ is absorbed and then re-excreted into the intestine, hardly merits discussion.

In dealing with the P_2O_5 metabolism, the experiments may be divided into six groups according to the CaO/P_2O_5 ratio and the P_2O_5 balance, as follows: group I, high $CaCO_3$ ration, P_2O_5 balance definitely positive, but low; group II, "balanced" $CaCO_3$ ration, P_2O_5 balance definitely positive; group III, stock ration, P_2O_5 balance definitely positive; group IV, stock ration *minus* fish meal, and *plus* medium Na_2HPO_4, P_2O_5 balance definitely positive; group V, stock ration *plus* high Na_2HPO_4, P_2O_5 balance definitely positive; and group VI, steamed bone flour and dicalcium phosphate ration, P_2O_5 balance very negative.

There are thus five groups, I to V, showing a positive P_2O_5 balance; and an outside group, group VI (placed where it is in Table II because of the CaO/P_2O_5 content of the ration), in which calcium phosphate is being fed, with a very definite negative P_2O_5 balance.

The results obtained in groups I and II show that, in spite of a fairly high $CaCO_3$ content of the ration, rendering P_2O_5 insoluble and unabsorbable, enough P_2O_5 has been taken up, as alkaline phosphates, to give rise to a positive balance: group V, on the other hand, demonstrates that a large positive balance of P_2O_5 may occur even when the food contains little CaO and indicates that P_2O_5 may be fixed in the body, as is easily understandable, largely in other forms than in association with CaO. The results of groups III and IV point, though in less degree, in the same direction.

The findings in group VI are of great interest. In considering them, the figures for rabbit *C* are not taken into account, for, although the results are of the same order, this rabbit, immediately preceding the experiment was on a high $CaCO_3$ ration, a circumstance reflected in the high negative CaO balance. The results in the two rabbits left in this group may be compared with those obtained in the rabbit in group II. The rations in the two groups have approximately the same CaO/P_2O_5 ratio, but the rabbits in group VI are consuming considerably more P_2O_5 than that of group II. Nevertheless, the P_2O_5 balances in group VI are markedly negative, while that in group II is positive. This is associated with the fact that the Ca and P in the ration of group VI are in the form of calcium phosphate while, in group II, they exist as $CaCO_3$ and food phosphorus, independent of one another. Results, somewhat similar in nature, were obtained by Tereg and Arnold in dogs [1883].

The same state of affairs is reflected in column X of Table II, where the amount of P_2O_5 passed in the urine is expressed as a fraction of the intake in the food. In group VI the negative balance has occurred in spite of the large intake of P_2O_5 and although a very considerable fraction of this intake has actually passed through the body of the animal and been excreted in the urine.

It seems therefore that, if a positive balance is to be regarded as evidence of utilisation, regard being had to the P_2O_5 and CaO balances in these groups, calcium attached to phosphorus in the form of calcium phosphate is of much less value to the animal economy than it is in circumstances where the Ca and P are supplied uncombined with one another, in the form of salts such as $CaCO_3$, $CaCl_2$ *etc.*, and Na_2HPO_4 *etc.* Because of the volatility of the H_2CO_3 of $CaCO_3$ on neutralisation and the consequent loss of acidity of the intestinal contents, salts of calcium with non-volatile acids would also appear to be preferable.

The moderate and approximately equal positive balances of both CaO and P_2O_5, obtained in group II, support the view, expressed in the introduction (*vide supra*), regarding the optimum conditions for ensuring the not too rapid simultaneous absorption of CaO and P_2O_5, namely as to their being present in moderate and approximately equivalent amounts.

Attention may be directed to the very large positive CaO balances in group I in connection with the subject of normal Ca and P metabolic activities of herbivora. The conditions present here, with the excretion of large quantities of calcium as $CaCO_3$ and small amount of phosphorus in the urine *etc.*, duplicate these to a considerable extent and need not be further described.

A few remarks may be made on the bearing of the results obtained in the present investigation on the subject of rickets. The characteristic finding in the blood in this disease is a considerable decrease in P in the serum [Iversen and Lenstrup, 1919; Howland and Kramer, 1921; Hess, 1930]. Shohl, Brown, Rose, Smith and Cozard [1931] have shown that an alkalotic condition of the blood occurs in rickets. This, together with an absolute deficiency of P in the diet or one produced by excess of calcium in the diet[1], would have a tendency to keep at a low level the P in the blood available for metabolism; while such curative and preventive measures as fasting and the administration of vitamin D [Kramer, Shear and Siegel, 1931; McGowan *et al.*, 1931], by supplying phosphate as well as acid endogenously, would, by keeping P in solution in quantity in the blood, have an opposite effect.

In the introductory discussion, allusion was made to the theory that $Ca_3(PO_4)_2$ circulates in a state of supersaturation in the blood, and the difficulties in accepting such a hypothesis were remarked on. It remains to state briefly how some of the difficulties especially in regard to calcification and decalcification may be overcome by the theory advanced in this communication. The vital activity of the osteoblasts would be capable of producing, locally where required, H_2CO_3 and H_3PO_4 for the precipitation of Ca from the $CaCl_2$, circulating in the blood under the protection of its proteins: while the osteoclasts, by reversing the process and breaking up $CaCO_3$ and $Ca_3(PO_4)_2$ of the bones, would supply $CaCl_2$ and NaH_2PO_4 to the blood, to be protected as before from interaction and kept in solution by the blood-colloids.

During the course of the investigation the Ca and P concentrations in the blood were estimated in certain cases. The taking of the samples could not be regulated with regard to the time of feeding as the animals consumed their ration at all times. As, however, the animals were bled at the same time each day—between 11 and 12 a.m.—and, as the routine of the experiments otherwise was identical, the results are roughly comparable. Table III supplies them, arranged in relation to the CaO/P_2O_5 ratio of the food eaten.

Table III shows that, while there may be a tendency for some of the animals on a diet with high CaO/P_2O_5 ratio to show a high Ca concentration in the blood and for the average to be higher than that of those on a low CaO/P_2O_5 ratio diet,

[1] This point will be discussed in a subsequent communication.

Table III.

No.	Rabbit	Date	Ca mg./ 100 cc.	P mg./ 100 cc.	CaO/ P_2O_5	Ca × P	Group	Diet
1	*C*	16. x. 31	16·7	2·81	2·2	46·92	I	High $CaCO_3$
2	*D*	25. ix. 31	13·1	3·96	—	51·87		,,
3	*H*	4. xii. 31	19·0	3·55	—	67·45		,,
4	*H*	10. xii. 31	16·3	3·39	—	55·25		,,
5	*E*	5. xi. 31	13·7	4·0	0·49	54·8	II	"Balanced" $CaCO_3$
6	*G*	4. xii. 31	14·5	3·75	0·50	54·37	VI	Tricalcium phosphate
7	*G*	10. xii. 31	18·2	3·55	—	64·61		,,
8	*G*	17. xii. 31	14·1	3·97	—	55·97		Dicalcium phosphate
9	*C*	7. x. 31	15·6	3·38	0·17	52·72	III	Stock
10	*D*	21. ix. 31	12·4	3·97	—	49·22		,,
11	*D*	23. ix. 31	12·5	3·93	—	49·12		,,
12	*D*	7. x. 31	13·4	4·07	—	54·53		,,
13	*G*	24. xi. 31	12·7	4·14	—	52·57		,,
14	*N*	2. xi. 31	15·1	3·07	—	46·35		,,
15	*D*	16. x. 31	13·1	4·22	0·04	55·28	IV	Stock less fish meal
16	*G*	18. i. 32	12·2	3·73	—	45·5		,,
17	*H*	18. i. 32	14·6	3·54	—	51·68		,,
18	*G*	26. i. 32	14·2	3·54	0·037	50·26		Medium Na_2HPO_4
19	*G*	8. ii. 32	12·3	4·16	0·0018	51·16	V	High Na_2HPO_4

the difference is not striking. There is no significant or consistent difference in the P concentration of the blood in the different groups. There is no evidence of the existence of a reciprocal relationship between the Ca and P concentrations, and the Ca × P product, varying as it does between 45·5 and 67·45, shows little sign of constancy, that is in absolutely healthy animals under identical conditions and with but slight differences in diet and indeed even when on the same diet.

Summary and conclusions.

The results obtained, in so far as they concern calcium in the ration, have special reference to conditions where calcium is supplied as $CaCO_3$. There seems to be no reason, however, why they should not be applicable, if in less degree, to situations where calcium is supplied in other forms.

The most important single factor controlling the absorption of Ca and P appears to be the acidity of the gastric juice: subject to this condition, the most important factor influencing the absorption of P seems to be an excess of Ca in the diet, which, having neutralised the HCl, precipitates P as $Ca_3(PO_4)_2$.

The optimum conditions for the simultaneous absorption of Ca and P seem to be those where, in the presence of adequate HCl, CaO and P_2O_5 exist in the diet in moderate and approximately equivalent amounts and in such quantity that the amount of Ca is considerably short of that required for the neutralisation of the HCl.

For the optimum utilisation of Ca and P after absorption, it seems to be requisite that they should be supplied in the food uncombined with one another and that they should be absorbed as far as possible in this condition.

The Ca and P salts of the blood seem to be prevented from interacting with one another and to be held in solution by the proteins; an "acid" condition of the blood, by increasing the solubility, will assist in this. High concentration of P in the blood is usually, in natural conditions, associated with an "acid" state; and here analysis will show Ca and P concentrations in various proportions.

Where, however, the reaction of the blood has an "alkaline" tendency, as occurs in normal and alkalotic conditions, chemical activity, more or less restrained previously, asserts itself and a balanced reaction occurs, with a point of equilibrium towards the side of the element—Ca or P—the salts of which are present in the greater concentration; and, as under such conditions $Ca_3(PO_4)_2$ is precipitated and calcium salts usually predominate, analysis will show a high concentration of Ca relatively to that of P.

Under such circumstances, there seems to be no warrant for the assumption that, in general, there exists a reciprocal or inverse relationship between the Ca and P concentrations of the blood.

Howland and Kramer's index of normality appears also not to be valid because, apart from the considerations just mentioned, the Ca × P product is not in fact a constant in normal individuals or even in healthy animals kept under fairly normal and identical conditions and fed on the same diet or on slight variations from this.

It is pointed out that the concentrations of P and Ca in the blood are the resultants of the factors of absorption, excretion and endogenous formation and fixation. If this is taken into consideration, along with the effect of the balanced reaction in "alkaline" conditions as just described, it will be seen how difficult it is to correlate Ca and P concentrations of the blood with other co-existent phenomena. The fact that the concentrations of Ca and P bear no relationship to the chemical activities of the elements as they are combined in the blood also requires emphasis in this connection.

Although the rapid absorption of quantities of Ca or P is followed by their rapid excretion in order that the composition of the blood may be kept within normal limits, considerable amounts of Ca may be fixed in the body in conditions where there is little P absorbed and *vice versa*.

The possible relation of the results obtained to a theory of the causation of rickets, namely deficiency of P, is discussed.

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