Studies in the Vitamin C Metabolism of the Pig*

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The detailed investigation of the composition of sow's milk carried out by Braude, Coates, Henry, Kon, Rowland, Thompson & Walker (1947) confirmed the earlier finding (Braude, Kon & Thompson, 1945–6) that sow's colostrum and milk are appreciably richer in vitamin C than the milk of most other species studied except the guinea-pig (see Table 1).

Table 1. *The concentration of vitamin C in the colostrum and milk of different animals*

Animal	Vitamin C: Colostrum (mg./100 ml.)	Vitamin C: Milk (mg./100 ml.)	Reference
Guinea-pig	—	29	Houston & Kon (1939)
Sow	25	12	Braude *et al.* (1945–6, 1947)
Mare	—	10	Holmes & Jones (1947)
Whale	—	7	Begg (1947)
Woman	—	4	Kon & Mawson (1950)
	7	6	Munks, Robinson, Williams & Macy (1945)
Ewe	5	0·8	Satterfield, Bailey, Foster & Hostetler (1942)
Cow	2	2	Kon & Watson (1937)
Goat	2	1	Richmond, Satterfield, Grinnels & Dann (1940)
Rat	—	0·4	Houston & Kon (1939)

The known ability of the adult pig to dispense with vitamin C in its food added interest to this finding, and it was considered of value to investigate some aspects of the vitamin C metabolism of both the adult and the sucking-pig to discover whether the richness of colostrum and milk in vitamin C was connected with an inability on the part of the young pig to synthesize vitamin C for its needs.

EXPERIMENTAL

General plan of experiment

Measurements with sows

To determine whether the secretion of vitamin C in high concentration into the colostrum and milk caused any marked change in saturation of the tissues of the sow, measurements were made with blood and urine of eight sows during the month preceding farrowing. During the week after farrowing the concentration in colostrum and milk was measured as well.

* Read in part before the Biochemical Society (Braude, Kon & Porter, 1948).

Metabolic experiments with piglets

Some 18 months after the experiments with sows, metabolic experiments were done with piglets removed from the sow at birth and reared in metabolic cages.

Urinary excretion of vitamin C. In the first series of experiments piglets were reared in pairs. One of each pair received vitamin C and the other did not. The urinary excretion of vitamin C was measured during 3 weeks and the vitamin C content of some body organs was determined at the end of the experiment.

Effect of chlorobutanol on urinary excretion of vitamin C. In similar experiments chlorobutanol was given by mouth to a number of piglets in an attempt to raise their urinary output of vitamin C, either by increasing synthesis or by depleting the body reserves. Several preliminary trials were necessary to establish that the highest non-anaesthetizing daily dose for the piglets was, per kg., 0·06 g. chlorobutanol, dissolved in 1 ml. arachis oil.

As before, pairs of piglets receiving, or deprived of, vitamin C were reared in the metabolic cages and their urinary excretion of vitamin C was determined. One of each pair was dosed with chlorobutanol, either daily or at intervals of a few days.

Vitamin C in blood and urine. During the course of the above experiments a model D.U. Beckman photoelectric spectrophotometer became available, making it possible to apply the micro-method of Lowry, Lopez & Bessey (1945) for the determination of vitamin C in small quantities of blood serum.

With this technique the first series of metabolic experiments was repeated and vitamin C was measured daily in the blood serum of two pairs of piglets.

Total vitamin C content of piglets. In the final series of experiments the change in the vitamin C content of piglets during the first 16 days of life was followed. Three pairs of piglets were taken from one litter. The first pair was killed at birth and macerated in a Waring Blendor with an equal weight of 5 % (w/v) metaphosphoric acid. The second pair was given for 6 days before killing a diet deficient in vitamin C and was then treated as the first pair. The third pair was kept on the same diet for 16 days before killing. Vitamin C was determined daily in the blood serum and urine of the piglets.

Methods

Sows

Breed, feeding and management. All the sows were of the Large White breed. The feeding and management were as previously described (Braude *et al.* 1945–6, 1947).

Collection of urine, blood, colostrum and milk. Samples of urine and of blood from an ear vein were collected in brown glassware 28, 14 and 7 days before the expected date of farrowing, at farrowing, and 2 and 7 days after farrowing. On the last three occasions samples of colostrum or milk were also collected as described by Braude *et al.* (1947).

Piglets

Management. Piglets were taken from the sow at farrowing. They were dried with a cloth and were placed in a basket after their teeth had been removed. After about 1 hr. the male piglets were weighed and those of approximately equal weight selected and transferred to the metabolism cages.

Metabolism cages. The cages, as illustrated in Pl. 1, were 2 ft. 3 in. long, 1 ft. 6 in. high and 1 ft. 9 in. wide. The sides and top were made from 10 s.w.g. wire formed to 1 in. diamond mesh, and the floors from 16 s.w.g. wire formed to 0·25 in. square mesh. The units were galvanized after manufacture. The cages were mounted on runners in wooden frames to allow easy removal for cleaning and sterilizing. Beneath each cage a vitreous-enamelled tray 2 ft. 6 in. long, 2 ft. wide and 1 in. deep was placed on runners with falls to one corner. A hole in the tray at this corner allowed urine to run through into a funnel connected to the container surrounded by melting ice in a vacuum flask.

Brown glass feeding bottles, fitted with an air inlet and with a bung carrying a glass tube reaching to the bottom were held by retort clamps as shown in Pl. 1 for use after the initial hand feeding. The teat was inserted through the wire mesh at a suitable height for the piglet, which could then help itself. It was sometimes necessary to place a small glazed bowl on the tray directly beneath the teat to catch any milk dribbled by the piglet during feeding and thus avoid contamination of the urine. The cages were kept in a temperature-controlled room at $15° \pm 2$.

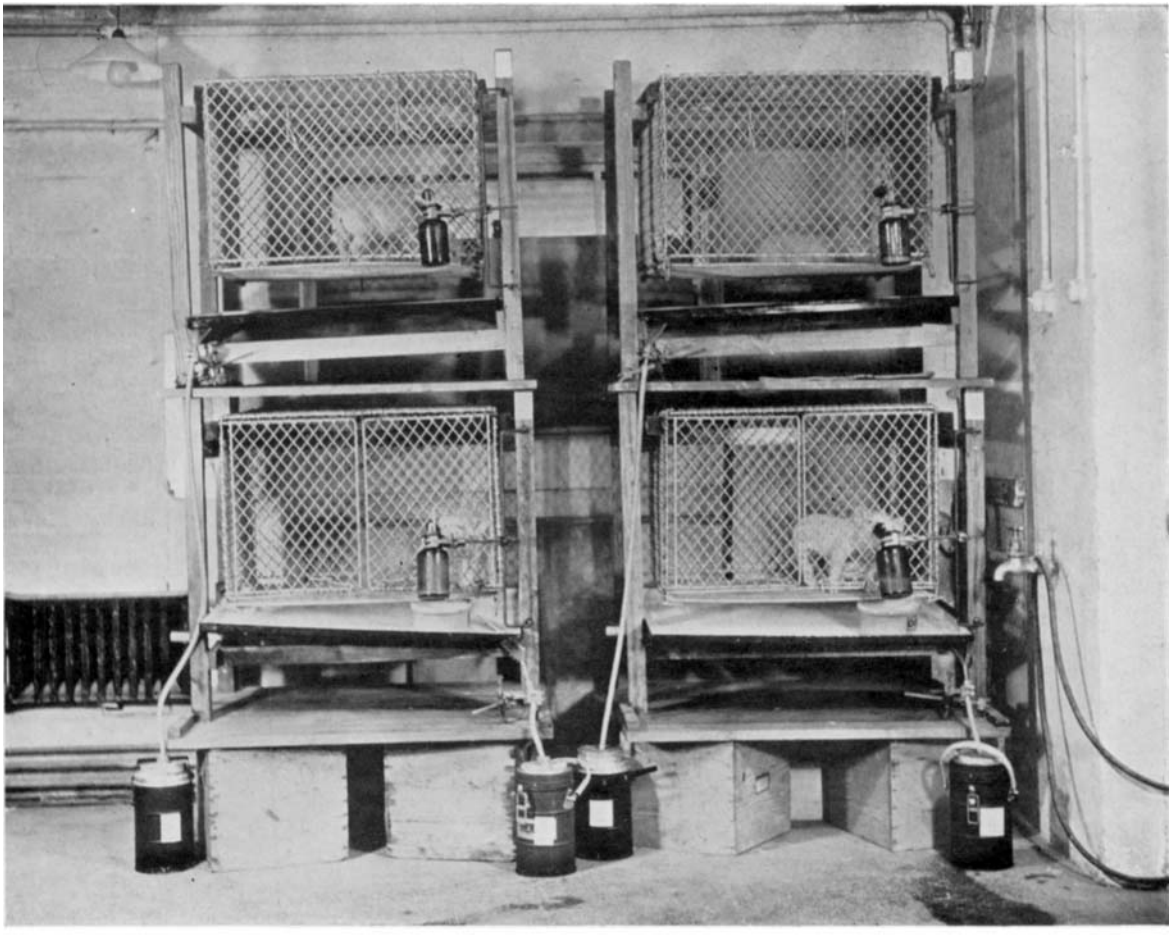
Collection of urine and blood. At each feed (see below) the container for collecting the urine was emptied into a large brown glass bottle kept at 2°. The total volume of urine voided each day was measured and sampled for analysis.

Blood samples, when required, were obtained by pricking an ear vein and collecting the resulting drop of blood in a thin-walled capillary tube. Serum was prepared by the method of Lowry & Hunter (1945).

Preparation of feed. Sow's colostrum was collected (see Braude *et al.* 1945–6), frozen and stored at $-25°$ until required. As it was not practicable to collect sufficient sow's milk for rearing the piglets, a substitute had to be found. In our experience goat's milk, with a composition more nearly that of sow's milk, was much more satisfactory than cow's milk, which tended to cause scouring.

Each day the colostrum, brought to room temperature, or milk was irradiated with a mercury-vapour lamp, after the addition of a riboflavin solution supplying 1 mg./l., to oxidize the vitamin C present. The colostrum or milk was placed in a flat, enamelled dish and stood 12 in. below a quartz mercury-vapour lamp (Thermal Syndicate, type TM/5/155). Owing to its opacity, the amount of colostrum irradiated at any one time had to be such that the depth of liquid did not exceed 0·5 in. With milk a depth of 1–1·5 in. was satisfactory. The liquid was stirred during irradiation to prevent formation of a skin on the surface. The colostrum required about 3 hr., and the milk 0·5 hr., for complete oxidation of the vitamin C. As the liquid became appreciably warm during the irradiation, much of the vitamin C was irreversibly destroyed, and after irradiation the liquid was heated to 40° for 10 min. to complete the destruction. The milk or colostrum was cooled and, on the assumption that the riboflavin originally present and that added had been destroyed by irradiation, more riboflavin was added to bring the concentration to 150μg./100 ml. The colostrum or milk was stored at 2° until used. Crystalline vitamin C was added to the colostrum (30 mg./100 ml.) and to the milk (12 mg./100 ml.) of those piglets allocated to the diet containing it.

Feeding. At first the piglets were fed with colostrum by hand from a brown glass



Metabolism cages for piglets

bottle fitted with a rubber teat. For the first 24 hr. they were fed at 2 hr. intervals, being allowed as much as they would take. Then, after two feeds of a 1:1 mixture of sow's colostrum and goat's milk, they received goat's milk. The interval between feeds was gradually increased to 3 hr. and finally the piglets were trained to feed themselves from the self-feeders. The milk in these was changed every 6 hr. and the bottles were thoroughly washed before refilling. The weight of milk consumed at each feed was recorded throughout the experiments. The piglets were weighed every day.

The piglets were not dosed with iron during the experiments as it was found that the unabsorbed iron salt excreted in the faeces interfered with the determination of vitamin C in the urine, which inevitably came in contact with the faeces during collection. The omission of iron, normally given during the 1st week of life to piglets reared indoors, led to their becoming anaemic towards the end of our experiments. We do not consider that this affected the results.

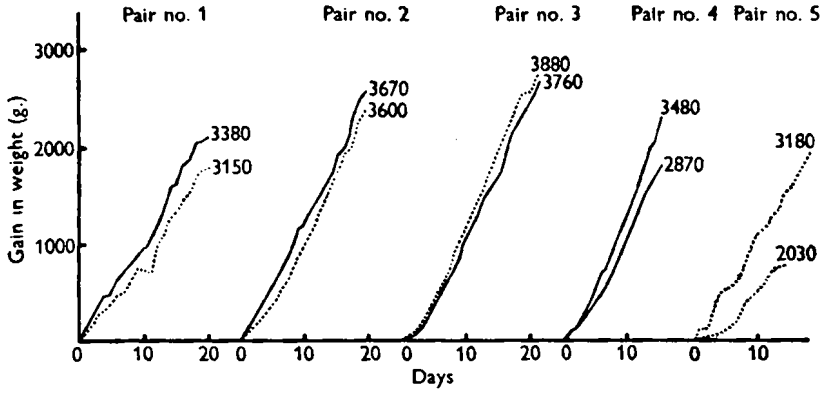


Fig. 1. Gain in weight of five pairs of piglets reared in metabolism cages. ——, piglets receiving vitamin C; - - - -, piglets deprived of it. The figure at the head of each curve shows the weight in g. at slaughter.

In general, the piglets grew satisfactorily in the metabolism cages. As an example Fig. 1 gives details of the growth of five pairs that behaved normally throughout the test period. The milk consumption of the piglets increased rapidly with age and it was necessary to restrict the amount drunk during the 2nd and 3rd weeks to avoid overfeeding which caused digestive disturbances.

The piglets grew at a rate only slightly below that of their litter-mates fed by the sow. Occasional piglets developed severe scours of unknown origin and had to be discarded.

Analytical methods

Vitamin C in colostrum and milk. Since samples collected as described contain only reduced vitamin C (Thompson, private communication) the following simplified method was used. Colostrum or milk (5 ml.) was added to 10 ml. of 5 % (w/v) metaphosphoric acid and the mixture was shaken and then stood for 10 min. before filtering. Portions (5 ml.) of the filtrate were titrated with standard 2:6-dichlorophenolindophenol.

Vitamin C in blood. For sow's blood, both direct titration with 2:6-dichlorophenol-indophenol of the filtrate from plasma (precipitated with 5 % (w/v) metaphosphoric acid) and the dinitrophenylhydrazone method of Roe & Kuether (1943) were used. Concordant results were obtained. With piglets, blood serum was used for the micro-modification of the dinitrophenylhydrazone method (Lowry *et al.* 1945). Good agreement was obtained between the micro-method and the two macro-methods.

Vitamin C in body tissues. The organ, or a suitable portion of it, was ground in a mortar with about twenty times its weight of 4 % (w/v) metaphosphoric acid. After filtration, portions of the filtrate were titrated with 2:6-dichlorophenolindophenol.

Total vitamin C content of piglets. Immediately after killing, the piglet was cut into small pieces and macerated in a Waring Blendor with its own weight of 5 % (w/v) metaphosphoric acid. Portions of the filtrate from the resulting slurry were titrated with 2:6-dichlorophenolindophenol.

Vitamin C in urine (chemical method). The method used was based on the selective oxidation-reduction method of Stewart & Sharp (1945). The results were in good agreement with those obtained by the biological assay with guinea-pigs, described below, but measurement by the methods for blood and milk, described above, gave consistently higher results.

Phosphate buffer (19 ml.) at pH 5·9, prepared by dissolving 30·65 g. of KH_2PO_4 and 8·95 g. of $Na_2HPO_4.12H_2O$ in 1 l. of water, was added to 20 ml. of urine and the mixture cooled to 1–5°. Cucumber juice (prepared by expressing the juice from cucumbers, thawed after freezing at −25°) (1 ml.) was added and the whole was kept at 1–5° for 15 min. Two 5 ml. portions were then withdrawn and placed in 50 ml. centrifuge tubes, each containing 1 ml. of an *Escherichia coli* (*Bacterium coli*) suspension (prepared as described by Stewart & Sharp, 1945), using *E. coli* (American Type Culture Collection no. 9492), and 1 ml. of 0·05 % (w/v) sodium cyanide. After incubation at 35° for 45 min., 5 ml. of 6 % (w/v) metaphosphoric acid were added to each tube before titration with the 2:6-dichlorophenolindophenol reagent (titration A). Two further 5 ml. portions of the material treated with cucumber juice were each added directly to 5 ml. of 6 % metaphosphoric acid, and then titrated with 2:6-dichlorophenolindophenol (titration B). The difference between titrations A and B represents the total vitamin C in the sample, the values taken being the mean of the two sets of titrations.

Biological assay of vitamin C in sow's urine. For the biological assay, the urine of several sows was examined by the method of Stewart & Sharp (1945), and 7·5 l. were collected during 1 week from the sow passing the richest urine. The urine was concentrated to a thick syrup by distillation under reduced pressure. The syrup was dried over phosphorus pentoxide in a vacuum desiccator to yield 370 g. of a brown gum. This was divided into a number of portions, weighing 1·2, 0·6 or 0·3 g., separately wrapped in waxed paper for storage in screw-capped bottles in the refrigerator. The method of assay was essentially that of Harris & Olliver (1942) as described by Braude *et al.* (1947). The test doses were sprinkled with sugar and fed to guinea-pigs from a nickel spatula. Most of the animals took the doses fairly readily after the first 2 or 3 days, but any suspected of not swallowing their complete dose were discarded. The vitamin C

standard was given at levels of 1·0, 0·5 and 0·25 mg. Dosing was continued for 14 days. On each dose level five or six animals survived the whole test with the exception of those given the lowest dose of standard, of which only three animals gave a response. The figure used in calculation was the gain in weight during the period of dosing. The lines of response proved sufficiently straight and parallel to justify calculation of potency by the common-slope technique. The calculated potency was 1·16 mg./g. concentrate, with true fiducial limits at $P = 0{\cdot}95$ of 0·76 and 1·91 mg./g. The value by the oxidation-reduction procedure was 1·36 mg./g., whereas direct titration with 2:6-dichlorophenol-indophenol and Roe & Kuether's (1943) method gave 5·4 and 2·4 mg./g. respectively.

RESULTS

Sows

Table 2 shows the vitamin C content of the blood and urine of eight sows at intervals before and after farrowing and also of colostrum and early milk.

Table 2. *The concentration of vitamin C in the blood plasma and urine of eight sows before, at, and after farrowing, and in colostrum and milk*

Sow no.	Material examined	Before farrowing (mg./100 ml.) 4 weeks	2 weeks	1 week	At farrowing* (mg./100 ml.)	After farrowing† (mg./100 ml.) 2 days	1 week
2260	Urine	1·0	2·8	2·2	2·4	1·9	—
	Blood plasma	0·24	0·72	0·83	0·30	0·44	—
	Colostrum	—	—	—	31·2	—	—
12	Urine	1·1	8·4	—	3·2	—	—
	Blood plasma	0·24	0·53	0·88	0·50	—	—
	Colostrum or milk	—	—	—	10·7	—	—
2354	Urine	6·7	4·5	1·9	2·0	1·3	1·9
	Blood plasma	0·60	0·77	0·40	0·46	0·58	0·54
	Colostrum or milk	—	—	—	9·4	17·6	15·4
2360	Urine	5·0	—	3·1	2·7	2·4	2·1
	Blood plasma	—	0·81	0·46	0·34	0·55	0·47
	Colostrum or milk	—	—	—	12·8	14·5	14·1
201	Urine	2·9	3·9	2·8	2·8	2·8	—
	Blood plasma	0·61	0·77	1·1	0·94	1·07	—
	Colostrum or milk	—	—	—	30·6	11·5	—
823	Urine	1·9	1·34	1·6	—	—	—
	Blood plasma	0·76	0·86	1·1	1·55	—	1·1
	Colostrum or milk	—	—	—	21·5	—	12·2
2359	Urine	6·7	4·1	3·5	2·5	—	2·1
	Blood plasma	1·06	0·83	1·5	0·89	0·69	1·0
	Colostrum or milk	—	—	—	24·1	8·4	8·4
1962	Urine	1·8	1·5	3·0	2·2	2·4	2·0
	Blood plasma	0·49	0·33	0·93	0·86	0·87	1·0
	Colostrum or milk	—	—	—	20·1	8·7	10·7
Mean	Urine	3·4	3·8	2·6	2·7	2·2	2·0
	Blood plasma	0·57	0·70	0·89	0·61	0·70	0·82
	Colostrum or milk	—	—	—	21·0	12·1	12·2

* Collected as described by Braude *et al.* (1945–6)
† Collected as described by Braude *et al.* (1947).

Piglets

Urinary excretion of vitamin C. The urinary excretion of vitamin C by three pairs of piglets during 3 weeks is shown in Fig. 2, and the results of the analyses of their organs in Table 3.

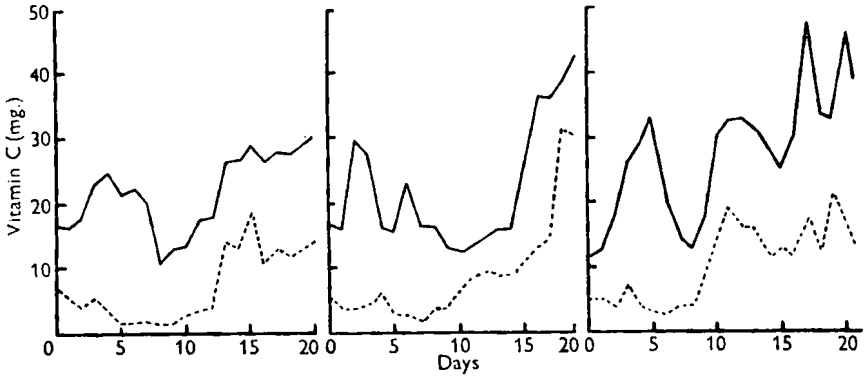


Fig. 2. Daily excretion of vitamin C in the urine by three pairs of piglets. ——, piglets receiving vitamin C; - - - -, piglets deprived of it. The first value is for the interval between birth and next 9.30 a.m.

Table 3. *The concentration of vitamin C in the blood plasma and in certain tissues of three pairs of 3-week-old piglets that received from birth, or were deprived of, vitamin C*

Piglets		Blood plasma	Liver		Adrenals		Spleen		Kidneys	
Type	No.	(mg./100 ml.)	Weight (g.)	Vitamin C (mg./g.)	Weight (g.)	Vitamin C (mg./g.)	Weight (g.)	Vitamin C (mg./g.)	Weight (g.)	Vitamin C (mg./g.)
Receiving vitamin C	1*a*	1·1	95	0·54	0·70	1·43	4·3	0·48	22	0·24
	2*a*	1·5	140	0·53	0·90	2·50	6·5	0·82	25	0·26
	3*a*	1·0	115	0·24	1·10	2·04	7·7	0·34	32	0·19
	Mean	1·2	117	0·43	0·90	1·99	6·2	0·55	26	0·23
Deprived of vitamin C	1*b*	0·28	105	0·19	0·85	1·33	3·5	0·33	22	0·13
	2*b*	0·91	150	0·29	0·65	—	4·2	0·48	30	0·20
	3*b*	0·34	139	0·11	0·80	1·69	8·3	0·18	34	0·08
	Mean	0·51	131	0·19	0·77	1·51	5·3	0·33	29	0·14

The weights of the piglets are given in Fig. 1.

The growth rates of these piglets (pairs nos. 1–3) are shown in Fig. 1, where the gain in weight is plotted against age.

Effect of chlorobutanol on urinary excretion of vitamin C. The results in Figs. 3 and 4 show the total urinary excretion of vitamin C by two pairs of piglets, one receiving it and one deprived of it, one of each pair being dosed with chlorobutanol as indicated. The vitamin C contents of the blood plasma and body tissues at the end of the experiment are shown in Table 4. The growth rates of these pairs (nos. 4 and 5) are shown in Fig. 1. Unfortunately the control piglet on the diet deficient in vitamin C developed bad scours and had to be killed before the end of the experiment.

Vitamin C in blood and urine. Fig. 5 shows the vitamin C level in the blood and the total urinary excretion of two pairs of piglets. One of each pair received vitamin C and the other did not.

Total vitamin C content of piglets. The total content and the total urinary vitamin C of each of the six piglets deprived of vitamin C are given in Table 5: the concentration in the blood serum is shown in Fig. 6.

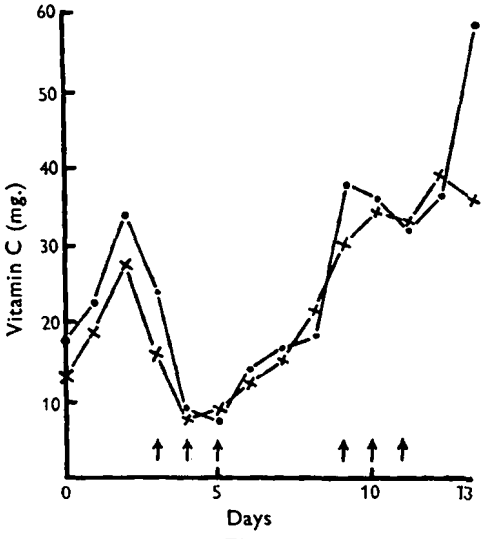


Fig. 3

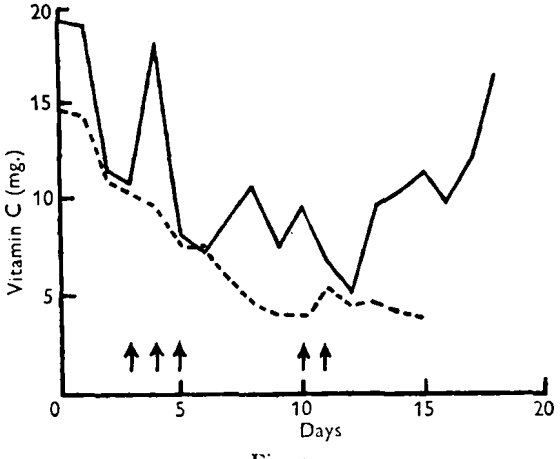


Fig. 4

Fig. 3. Daily excretion in the urine of vitamin C by two piglets receiving it. ●——●, piglet dosed with chlorobutanol (0·06 g./day) as indicated ↑ ; ×——×, undosed piglet. Timing as in Fig. 2.

Fig. 4. Daily excretion in the urine of vitamin C by two piglets deprived of it. ——, piglet dosed with chlorobutanol (0·06 g./day) as indicated ↑ ; - - - -, undosed piglet. Timing as in Fig. 2.

Table 4. *Vitamin C content of blood plasma and of certain tissues of one pair of piglets given vitamin C from birth, and of a deficient piglet. One of the pair and the other piglet were dosed with chlorobutanol as indicated in Figs.* 3 *and* 4

	Diet deficient in vitamin C Piglet		Diet with vitamin C Piglet	
Tissue	Undosed	Dosed with chlorobutanol	Undosed	Dosed with chlorobutanol
Blood plasma (mg./100 ml.)	Died from scours at 14 days	0·43	1·13	1·40
Liver (mg./g.)		0·19	0·52	0·28
Kidneys (mg./g.)		0·12	0·23	0·39
Spleen (mg./g.)		0·14	0·40	0·39
Adrenals (mg./g.)		1·22	1·80	2·44
Whole intestine (mg./g.)		0·14	0·35	0·35

The ages of the piglets are given in Figs. 3 and 4.

DISCUSSION

Sows

The results in Table 2 show no definite trends for the vitamin C levels in either blood plasma or urine, which thus seem uninfluenced by the high output of vitamin C in colostrum and milk. Grummer, Whitehair, Bohstedt & Phillips (1948) measured the

vitamin C in the blood of nine sows on each day between the 2nd and the 5th before farrowing, and on the 3rd day afterwards. They also found that farrowing had little effect on the plasma level of vitamin C, though their mean value of 0·36 mg./100 ml.

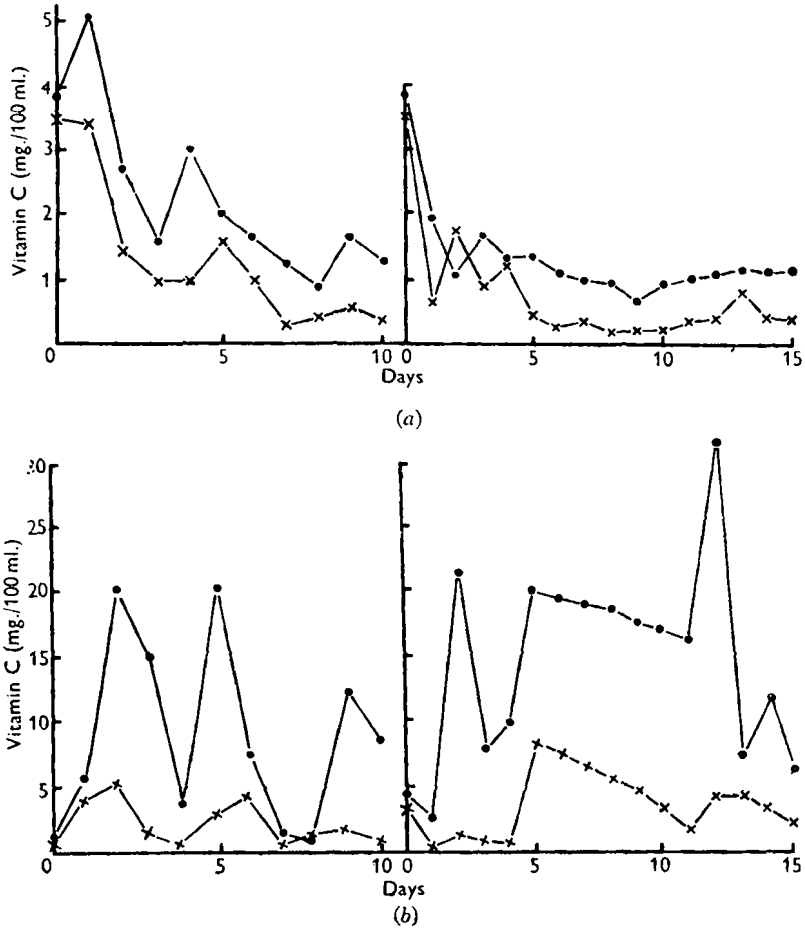


Fig. 5. (*a*) Concentration of vitamin C in the blood serum of two pairs of piglets. ●——●, piglets receiving vitamin C; ×——×, piglets deprived of it. (*b*) Daily excretion of vitamin C in the urine by the same two pairs of piglets. Timing as in Fig. 2.

Table 5. *Total vitamin C content of piglets at birth and total content of, and total excretion in the urine by, piglets* 6 *and* 16 *days old given from birth a diet deficient in vitamin C*

		Vitamin C		
		Piglet		Urine,
Piglet no.	Age when killed (days)	Total (mg.)	Concentration (mg./g.)	total excretion (mg.)
1	0	130	0·122	0
2	0	152	0·145	0
3	6	137	0·069	34
4	6	151	0·079	28
5	16	183	0·053	82
6	16	305	0·065	95

plasma, with a range of 0·06–1·09 mg./100 ml., was appreciably lower than the mean of 0·80 (range 0·30–1·55) observed during the equivalent period of the present study.

The high concentration of vitamin C in the colostrum and milk of sows makes it of interest to compare its concentration in the blood plasma with that in other species. Table 6 shows that the concentration of vitamin C in the plasma of sows falls within the normal range for those other species that synthesize their own requirement of the vitamin.

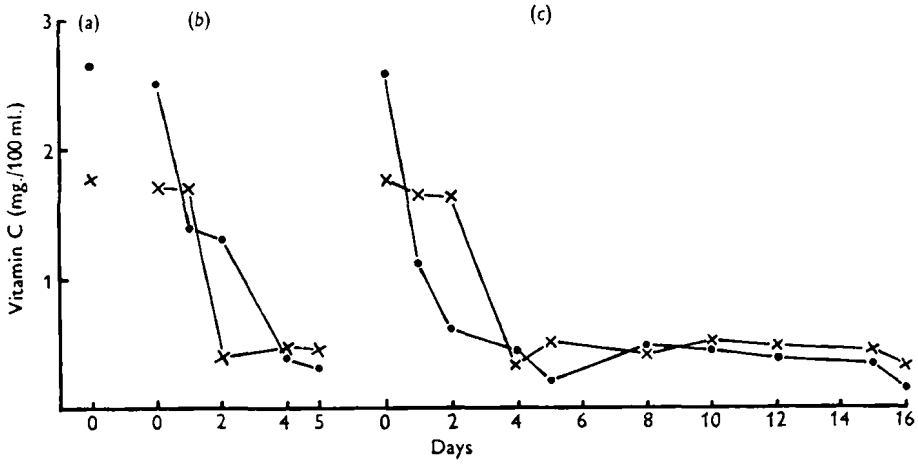


Fig. 6. Concentration of vitamin C in the blood serum of three pairs of piglets from one litter deprived of it. Pair (*a*) at birth; pair (*b*) during the first 5 days; pair (*c*) during 16 days.

Table 6. *Rounded off values for the concentration of vitamin C in the blood plasma of various animals*

Animal	Concentration (mg./100 ml.)	Reference
Dog	0·3	Munsell, Cuadros & Suárez (1944)
Rat, male	0·3	Todhunter & McMillan (1946)
Rat, female	0·9	Todhunter & McMillan (1946)
Cattle	0·4	Bortree, Huffman & Duncan (1943)
Rabbit	0·4	Munsell *et al.* (1944)
Goat	0·5	Munsell *et al.* (1944)
Horse	0·5	Munsell *et al.* (1944); Rasmussen, Cole & Miller (1944)
Sheep	0·6	Rasmussen, Cole, Miller & Thorp (1944)
Pig	0·7	Present study
Poultry	2·0	Satterfield, Bell, Cook & Holmes (1945)

It is more difficult to compare the concentration of vitamin C in the urine of sows with that in the urine of other animals, since fewer data are available. Moreover, the concentration of vitamin C in the urine normally varies much more from day to day than the plasma concentration. Knight, Dutcher, Guerrant & Bechdel (1941) report values of 2–5 ml./100 ml. in the urine of dairy cows, though the figures of Moore & Cotter (1945) and Vavich, Dutcher, Guerrant & Bechdel (1945) for the total daily output, 26–110 and 57 mg., would suggest a considerably lower concentration. Rats given a stock diet excreted 1 mg./day (Sutton, Kaeser & Hansard, 1942) corresponding to a concentration of approximately 10 mg./100 ml.

Piglets

The results in Fig. 2 for three pairs of piglets show that those piglets given a diet free from vitamin C excreted small amounts of the vitamin during the first 8–10 days of life, that a pronounced increase in excretion then occurred and the subsequent excretion, although not constant, was markedly greater than that during the first period. The piglets given vitamin C excreted substantial quantities of it during the first few days when they received colostrum; the excretion then fell but increased again at about the same time as in the deficient piglets.

Analysis of tissues (Table 3) showed that, with the exception of the adrenals, where the content was similar for all piglets, the tissues of the deficient animals contained only about half as much vitamin C as those of the piglets given vitamin C.

The marked increase in the total urinary excretion of vitamin C occurring about the 8th–12th day in both deprived and dosed piglets, together with the initially low urinary excretion of the deficient piglets, suggested either that vitamin C synthesis does not begin until the piglet is about 1 week old or that the requirement for vitamin C changes markedly at this stage.

The experiments with chlorobutanol were meant to decide between these alternatives. If a piglet could be induced to excrete a greater than normal amount of vitamin C during the 1st week of life, its body stores might become depleted, resulting, if no synthesis occurred, in clinical signs of vitamin C deficiency. However, as is evident from the results shown in Figs. 3 and 4, chlorobutanol had no effect on the urinary excretion of vitamin C whether or not the piglet was receiving it; the vitamin C content of the body tissues was also unaffected (Table 4). The finding about the urinary excretion, which is in marked contrast to the known effect of chlorobutanol in rats (Longenecker, Musulin, Tully & King, 1939), cattle (Bortree, Huffman & Duncan, 1943) and sheep (Colby, Lindley, Warwick & Cunha, 1948), was confirmed by an experiment in which a 6-month-old fattening pig was dosed with chlorobutanol (2 g.) and the vitamin C content of its urine determined at intervals during the following 72 hr.; no change was observed. The validity of our technique was tested in an experiment in which a rat was given chlorobutanol (0·03 g.); an increase of almost one hundredfold in the urinary concentration of vitamin C resulted.

Measurement of the level of vitamin C in the blood serum of pairs of piglets showed (Fig. 5) that there was no increase corresponding to that in urine at the 8th–12th day. At birth the level was relatively high, 3–4 mg./100 ml.; it fell to 1·5 mg./100 ml. during the next 3–4 days, remaining thereafter at 1·2 mg./100 ml. for the piglets receiving vitamin C, and at 0·5 mg./100 ml. for those deprived of it.

Grummer *et al.* (1948), who present data for the vitamin C in the blood serum of the sucking-pig, record an average value of 0·69 mg./100 ml. plasma at birth, rising to 1·5 mg./100 ml. after the first feeds from the sow and thereafter falling to 1 mg./100 ml. at the end of 1 week. Their findings agree in general with ours for the piglets given vitamin C, except that the content at birth was appreciably higher in all the piglets we examined.

The total output of vitamin C in the urine tended to follow the changes in the blood-

serum level during the first few days. Furthermore, our data show that the piglets deprived of vitamin C maintained in their blood a concentration sufficient to rule out the appearance of signs of deficiency. It seemed probable, therefore, that a piglet is able to synthesize vitamin C from birth since, unless the body reserves at birth are very large, it is unlikely that in the absence of synthesis the concentration in the blood could be maintained at 0·5 mg./100 ml., with an unknown quantity being metabolized and, in addition, some 1–5 mg. being daily excreted in the urine.

In experiments in which the total content of vitamin C of piglets was determined (Table 5) those killed after 6 days contained as much as their litter-mates at birth; moreover, they had excreted during this period about 30 mg. and metabolized a further, necessarily undetermined, amount. During a further week the piglets excreted about 60 mg. of vitamin C; the concentration in their tissues remained fairly constant but, as they nearly doubled their body-weights, the total quantity in the body increased twofold.

It is evident, therefore, that the piglet is able from birth to synthesize vitamin C. During the first week the requirement is probably high, resulting in low urinary excretion and a general fall in the tissue concentration, which remains fairly constant after this period, whereas the urinary output rises, suggesting that the period of high requirement has ended.

The high concentration of vitamin C in sow's colostrum and milk no doubt ensures that the young pig receives plenty of the vitamin during a period when it apparently metabolizes it at a high rate, but we have no proof that it really needs such a plentiful supply. In fact, though a teleological explanation of this high concentration in colostrum and milk seems attractive, it may be that some peculiarity in the metabolism of vitamin C by the lactating sow results in the synthesis of more of the vitamin than the sow needs, so that colostrum and milk provide an additional excretory pathway by which this excess is eliminated.

SUMMARY

1. Because sow's colostrum and milk are rich in vitamin C (Braude *et al.* 1945–6, 1947), and because the adult pig is known to be independent of an exogenous supply of the factor, experiments were done to determine the sucking-pig's ability to synthesize it.

2. The mean concentration of vitamin C in the blood plasma and urine of eight sows, 4, 2 and 1 week before farrowing, at farrowing and at 2 days and at 1 week afterwards, was 0·74 mg./100 ml. plasma (range 0·24–1·55) and 2·9 mg./100 ml. urine (range 1·0–6·7). The mean concentrations in colostrum and milk were 21 mg./100 ml. (range 9·4–31·2) and 12·2 mg./100 ml. (range 8·4–17·6).

3. Farrowing did not cause any marked change in the blood-plasma level or in the urinary excretion of vitamin C.

4. In metabolic experiments, pairs of piglets reared in metabolism cages received sow's colostrum followed by goat's milk, one of each pair receiving, and the other being deprived of, vitamin C. The piglets grew satisfactorily.

5. The vitamin C excreted in the piglets' urine was measured daily by the method of Stewart & Sharp (1945), which was proved by biological tests with guinea-pigs to be adequate for this purpose.

6. Piglets receiving, and deprived of, vitamin C excreted, respectively, during the first 2 days, 10–20 mg. and 2–5 mg.; during the next 6–10 days, 5–10 mg. and 2–5 mg.; and after 8–12 days, 20–30 mg. and 10–20 mg. This finding suggested either that the piglet has a high requirement for vitamin C during the 1st week of life, or that it does not start to synthesize the vitamin until about 1 week old.

7. Chlorobutanol was given to piglets in unsuccessful attempts to increase the urinary excretion of vitamin C.

8. The concentration of vitamin C in the blood serum of ten piglets at birth was 3–4 mg./100 ml. The level fell during the 1st week and then remained at about 1·0 mg./100 ml. for two piglets receiving vitamin C and at about 0·5 mg./100 ml. for the six deprived. There was no increase in the blood level corresponding to that in the urine at the 8th–12th day.

9. The total amounts of vitamin C were measured in pairs of piglets at birth and after 1 and 2 weeks on a diet devoid of it. The mean values, 140, 140 and 245 mg., show that the piglets could synthesize vitamin C from birth and suggest that the low urinary excretion observed during the 1st week was probably due to their high requirement for vitamin C at this age.

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A Constant-Temperature Room for Small-Scale Experiments with Young Chicks

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Under artificial conditions very young chicks must be provided with supplementary heating and protection from draughts. In the many types of commercial brooder these requirements are usually met by screens and heaters. The temperature in the screened compartment is normally kept at 95° F. for the 1st day or two after hatching and is then gradually reduced to 80° F. during the following 3 weeks, after which auxiliary heating can be discontinued provided the room temperature is not abnormally low. Commercial brooders are usually designed to house about 100 chicks in each compartment. They can, however, be subdivided into sections, and for the normal work of this laboratory with groups of about twenty chicks these brooders, slightly modified, have provided suitable accommodation. For pilot experiments, or where the test material is scarce, much smaller groups of two or three chicks can be used with advantage, but difficulties were experienced in housing such small groups satisfactorily. They did not thrive in subdivided brooders and the risk of chill was considerably increased by the absence of the body-heat generated by larger numbers of chicks.

It therefore seemed desirable to construct a room in which the inside temperature could be held sensibly uniform at any selected value within the range normally used for chick experiments. The birds could then be housed in ordinary small-animal cages and as nearly as possible would have common conditions of temperature, light and ventilation. These conditions could be altered with the age of the chicks. The essential requirements of such a room appeared to be: (1) thermostatic temperature control with a range from normal ambient temperature to 95° F.; (2) temperature variation within the room limited to $\pm 2^{\circ}$ F.; (3) adequate and adjustable ventilation; (4) air velocities in the vicinity of the chicks limited to 40 ft./min.; (5) smooth and impervious internal surfaces for easy cleaning; (6) provision of uniform artificial lighting with suitable controls.

No provision was made for the control of humidity as it was assumed that close adjustment of this would not be a critical factor, an assumption justified by the recent work of Barott & Pringle (1949), demonstrating that chicks are unaffected by variations of humidity from 35 to 75 %.