91. THE EXCRETION OF VITAMIN A IN URINE¹

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(Received 4 July 1941)

SINCE vitamin A is insoluble in water one would not expect to find it in urine. As far as normal human urine is concerned this expectation appears to be correct. An early claim by Cooper [1924] to have detected vitamin A in human urine by biological means was criticized by Rowntree [1930], who could not confirm the presence of the vitamin in urine from children even when the diet contained milk, eggs, carrots and cod-liver oil. Kaufmann & Drigalski [1933] obtained negative results from human urine by colorimetric methods. In the urine of rats Davies & Moore [1934] found no vitamin A by the colorimetric method even when toxic overdoses of the vitamin were given. Przedziecka [1935] claimed to have detected vitamin A in urine by a colorimetric method, which was, however, applied in so unusual a manner as to leave doubt as to the significance of the observation.

In urine from pathological subjects, however, Boller & Brunner [1936] obtained positive results by the SbCl_3 method in 10 out of 42 cases examined. Half of the patients excreting the vitamin had cancer. Later, Boller *et al.* [1937] published results on 321 cases. Vitamin A was found in the urine in icterus with closure of the biliary duct, chronic nephritis, nephrosis, lobar pneumonia before crisis and cirrhosis of the liver. Large oral doses of vitamin A had little effect on the amounts excreted in the urine. Excretion was reduced by pyramidone.

The presence of vitamin A in pathological urine was confirmed by Schneider & Weigand [1937, 1, 2]. Excretion was observed in cancer, tuberculosis and chronic infections. The excretion was not due to specific renal damage, nor was it caused by increased ingestion of the vitamin. It was claimed that excretion only took place in hypovitaminosis C, and that it could be checked by giving ascorbic acid. Lindqvist [1937] found that in pneumonia the excretion of vitamin A varied from 228 to 3060 i.u. daily before crisis, falling to zero after crisis. Urinary excretion was associated with a lowered vitamin A content of the blood, which returned to normal after crisis. Gaetgens [1937] reported the presence of vitamin A in urine from 8 out of 31 cases of normal pregnancy. After large doses of vitamin A had been given it was found in the urine in 19 cases.

In chronic nephritis Hedberg & Lindqvist [1938] observed constant or irregular excretion of vitamin A in 23 out of 25 cases. In contrast with the finding in pneumonia the vitamin A content of the blood was often high. In livers taken at autopsy the reserves were frequently low, but bore no relation to

¹ A preliminary communication on this subject has been given to the Biochemical Society [Lawrie *et al.* 1938].

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the levels found in the blood. Large doses of vitamin A, ascorbic acid or pyridone did not effect urinary excretion. Of 26 patients examined by Grant [1938], 15 showed spontaneous excretion of vitamin A. These included patients suffering from carcinoma of the gall bladder, cirrhosis of the liver, icterus, hemeralopia, chronic nephritis, nephrosis, diabetes and pneumonia. The mean vitamin A content of the blood in this group was much lower than in the remaining group of 11 cases which did not excrete vitamin A, although the same diseases were often found in both groups. The low values for the excreting cases could not, however, be ascribed directly to loss in the urine, since some cases which were not excreting vitamin A showed equally low levels in the blood. The cholesterol content of the blood was independent of either the level of vitamin A in the blood or of the presence or absence of urinary excretion of vitamin A.

The finding of Hedberg & Lindqvist [1938] that reduction of vitamin A in the blood does not necessarily occur in cases of renal disease is supported by Catel [1937; 1938] who found that the blood vitamin A remained normal in children with nephritis accompanied by urinary excretion of the vitamin. This worker made the surprising claim, which has been confirmed in the present work, that vitamin A is present in the urine of the normal dog. Vitamin A was not detected in the urine of rabbits. Chevallier & Manuel [1938] have claimed that vitamin A may be present in the urine of guinea-pigs which have been given large doses of the vitamin.

The urinary excretion of vitamin A in skin diseases has been investigated by Marchionini [1938]. Out of 75 cases of non-tuberculous skin disease vitamin A was excreted spontaneously in the urine in 7 cases, and in 15 cases after massive doses of vitamin A. In 27 cases of tuberculous skin diseases, mostly lupus, spontaneous excretion was found in 1 case, excretion after dosing in 7 cases. In 14 cases of syphilis, with and without skin lesions, vitamin A was excreted spontaneously in 2 cases and after dosing in 9 cases. In subjects excreting vitamin A the blood vitamin A was sometimes low, but not always. No evidence of kidney damage was obtained, but in many cases the galactose test revealed reduced liver efficiency. The excretion of vitamin A appeared to be secondary to this injury. Erythema induced by ultra-violet irradiation caused reduction in the carotene and vitamin A contents of the blood, but did not result in the urinary excretion of vitamin A.

Recently, evidence to connect urinary excretion of vitamin A with injury or blockage of the reticulo-endothelial system has been advanced by Thiele and his colleagues. Thiele & Seedorf [1939] reported that vitamin A is occasionally excreted by patients undergoing treatment for gonorrhoea and tertiary syphilis. The excretion did not appear to depend on injury to the liver or kidneys, but was frequent in subjects treated by bismuth or by infection with malaria. Similar findings were recorded in a later paper by Thiele & Nemitz [1939]. The efficiency of the reticulo-endothelial system in subjects showing urinary excretion of vitamin A was tested by Thiele & Klodwig [1939]. When Congo red was injected into the blood stream its rate of disappearance was usually abnormally slow, indicating impairment of the reticulo-endothelial cells.

EXPERIMENTAL

Part I. *The examination of human and animal urines for vitamin A*

Method. 100 ml. of urine were shaken with successive additions of 5 ml. of saturated aqueous KOH, 10 ml. of ethanol and 100 ml. of ether. With urines containing much vitamin A smaller volumes of urine and reagents were some-

times taken. The ether layer was washed with water, evaporated and dissolved in 1 ml. of CHCl_3 . Vitamin A was estimated by the SbCl_3 method. To convert blue units into International Units a factor of 0.6 has been used [Moore, 1937].

Normal human subjects. Urine was collected from 6 subjects, of ages 7 months to 41 years. In no instance could even a trace of vitamin A be detected. The vitamin was not excreted by some of the same individuals when suffering from severe colds. One subject took halibut-liver oil to the extent of 30,000 I.U. of vitamin A daily for 2 months without causing urinary excretion. Another ingested 200 g. of raw ox liver, again with negative results.

Pathological human subjects. 83 urines from various medical cases were examined. The results are shown in Table 1. Vitamin A was excreted most frequently and in highest concentration in pneumonia. Next in order of frequency

Table 1. *The urinary excretion of vitamin A in human diseases*

The numbers after each disease which are not included in brackets give the vitamin A content per 100 ml. of urine for individual cases. The numbers in brackets give the corresponding daily excretions of vitamin A.

Frequent excretion of vitamin A

Respiratory diseases: Pneumonia 0, 0, 6 (126), 12 (37), 19, 60, 68, 105, 160 (3200), 250, 340 (1500), 340, 400, 400, 450, 480. Empyema 0, 3, 68. Pulmonary tuberculosis 0, 018 (pyonephrosis + calculus). Asthma 0, 15. *Kidney diseases:* Chronic nephritis 0, 0, 0, 0, 12 (96), 14, 18, 26, 37, 52. Sub-acute glomerular nephritis 34 (187). Acute nephritis 0, 0. Prostate uraemia 41. Nephrosis 68. Nephrosis with osteomyelitis 8. Albuminuria 0.

Infrequent or absent excretion of vitamin A

Rheumatic diseases: Rheumatic fever 0, 0, 9. Acute rheumatism 0. Muscular rheumatism 0. Rheumatoid arthritis 0, 0. Fibrositis 0. *Heart diseases:* Cardiac failure 0, 0. Aortic regurgitation 0, 0, 0. Hypertension 0. Coronary thrombosis 0. *Diabetes:* 0, 0, 0, 4, 5. *Cancer:* Obstructive jaundice due to carcinoma at head of pancreas 0. Carcinoma of colon 0. *Cerebral tumour:* 0, 0, 0, 0. *Liver diseases:* Cirrhosis of liver 0. Sub-acute atrophy 0. *Thyroid diseases:* Rheumatic carditis and hyperthyroidism 0. Thyrotoxicosis 0. *Miscellaneous:* Plummer-Vinson syndrome 0. Post-encephalitic Parkinsonism 0. Disseminated sclerosis 0. Splenic anaemia 0. Haemophilia 0. Pernicious anaemia 3. Haematemesis 0, 0. Abdominal tumour of unknown origin 0. Duodenal ulcer 0. Epilepsy 0. Abortus fever 0. Osteoarthritis 0. Dermatitis 0. Pyrexia of unknown origin 0.

of excretion and concentration was chronic nephritis. We have not noticed more frequent or pronounced excretion in any one type of pneumonia or chronic nephritis than in others. Excretion was fairly common in other respiratory and kidney diseases. In our remaining 44 cases of general diseases we found only three instances of the excretion of small amounts of vitamin A. No significance need be attached to our failure to confirm the positive results obtained by previous workers for cancer and skin diseases in view of the small number of cases which we have examined. In pneumonia we found, in agreement with other workers, that the excretion of vitamin A ceased abruptly when the crisis had been passed. In chronic nephritis the excretion was usually continuous and permanent.

Pregnancy in women. Samples of urine from 32 cases of pregnancy were obtained through the kindness of Drs H. R. Youngman and W. B. Hedgcock. The duration of pregnancy had varied from 16 to 38 weeks. Of 30 subjects who received no special vitamin A therapy, 24 were described as normal. In only one instance was a trace of vitamin A detected in the urine. No vitamin A was detected in urine from two cases of urinary infection. Vitamin A was present in

the urine in one out of four cases of albuminuria, to the extent of 12 I.U./100 ml. Urine from a normal subject who had received 12,000 I.U. daily of a vitamin A concentrate for a month, and from a subject, recently recovered from urinary infection, who had received halibut-liver oil to the extent of 70,000 I.U. daily for 1 week, gave negative results.

These results suggest that the urinary excretion of vitamin A in pregnancy, at least in this country, is less prevalent than the data of Gaetgens [1937] would indicate for Germany.

The excretion of vitamin A by animals

Dog. Catel [1937; 1938] has reported that vitamin A is present in the urine of the normal dog. We have confirmed this, using urine from a healthy male fox terrier. Many specimens were examined and they were always positive. The concentration of vitamin A varied between 90 and 450 I.U./100 ml. of urine, determined colorimetrically, the latter value being equal to the highest concentrations we have found in urine from patients with pneumonia. The diet of the dog normally included household scraps, biscuits and occasional liver. When the animal was deprived of liver and greens for 10 days and then restricted for 3 days to a diet low in vitamin A (biscuits only) there was no diminution in the excretion of vitamin A. The restoration of a mixed dietary for the next 3 days, in conjunction with the administration of vitamin A (120,000 I.U. daily as halibut-liver oil) was associated with a marked decrease in the concentration of vitamin A in the urine. While we are inclined to attribute this anomalous observation to mere coincidence, it seems clear that vitamin A as ingested cannot pass freely into the urine, and that the amount of vitamin A excreted is determined by factors other than the supply immediately available from the diet.

Rabbits. Catel [1937; 1938] could not detect vitamin A in the urine of rabbits. We have confirmed this finding for normal adult rabbits, and have found only small traces of the vitamin in the urine after infection with sputum from a human patient with pneumonia.

Our first rabbit was given a subcutaneous injection of a suspension of sputum in the groin. The concentration of vitamin A in the urine during the next 2 days was only *ca.* 10 I.U./100 ml. The animal then died, the autopsy revealing red and grey hepatization of the lungs and acute congestion of the spleen, liver and kidneys. A second rabbit was similarly treated and died after 3 days. Bacterial examination showed the presence of pneumococci in the blood and lungs. Only a trace of vitamin A (2 I.U./100 ml.) was present in the urine. In order to rule out the possibility that the failure of the first two rabbits to excrete large amounts of vitamin A had been due to the inadequacy of the reserve in their liver a third rabbit was given a diet rich in green vegetables, with 5 drops of cod-liver oil per week for nearly 2 months. During the last 5 days before the injection of sputum a total of 25,000 I.U. of vitamin A was given as halibut-liver oil. No vitamin A could be detected in the urine either before or after the injection. At autopsy the liver was found to contain a total of 17,000 I.U. of vitamin A, equivalent to 210 I.U./g.

In view of the report of Thiele & Seedorf [1939] that treatment with bismuth may cause the excretion of vitamin A, rabbit no. 3, before receiving the injection of pneumococci, was given a single injection of 25 mg. of metallic bismuth as a watery suspension (Bismoid, Eli Lilly and Co.). No excretion of vitamin A could be detected during the next 48 hr., although the presence of bismuth in the urine could readily be demonstrated by chemical tests.

Rats. Vitamin A was not detected in the urine of either normal or diseased rats. The injection of pneumonia sputum into rats neither affected the health of the animals nor caused excretion of vitamin A. An attempt to infect the animals with pus taken from the kidney of another rat was also unsuccessful. Repeated bismuth injections over a period of 8 days did not cause excretion of

the vitamin. No vitamin A was found in urine collected from rats having severe degeneration of the epithelium of the convoluted tubules of the kidneys caused by prolonged deficiency of vitamin E [Martin & Moore, 1939], nor in the urine of a rat in the final stages of a fatal respiratory disease of spontaneous origin.

Biological and spectroscopic confirmation of the presence of vitamin A in urine.

Previous workers have relied on the SbCl_3 test for the demonstration of vitamin A in urine extracts. Boller *et al.* [1937] reported that the absorption band in this reaction is at about $620\text{ m}\mu$, the position characteristic of the vitamin. We have confirmed this observation. It seemed desirable nevertheless to rule out the possibility that the SbCl_3 reaction might be simulated by some unidentified excretory product. An extract of urine from a patient with empyema, who excreted about 70 I.U. of vitamin A/100 ml. according to the SbCl_3 test, was therefore examined in the ultra-violet spectrophotometer. A shallow band at $328\text{ m}\mu$ was observed. Biological tests were made with extracts of urines from a patient with pneumonia and from the healthy dog, which contained 180 and 150 I.U. of vitamin A/100 ml. respectively. In each instance increases in weight were observed when the extracts were dosed to rats at levels equivalent to about 8 I.U. per day.

Part II. *The state of combination of vitamin A in urine*

Normal human urine is almost free from lipoids [Neuberg, 1911]. In disease fatty material may appear in the urine in one or other of the following forms: (1) fat globules or tallow-like particles floating on the surface, (2) a milky emulsion (chyluria), (3) fatty concretions and needle-shaped crystals, (4) constituents of cells rich in fat, (5) a lecithin-globulin complex giving rise to fine turbidity. Most of the urines containing vitamin A which we have examined do not fall into any of the above groups. Some were as clear as normal urine. Others were turbid, but on filtration many gave filtrates still containing vitamin A, yet as clear as freshly voided normal urine. Since vitamin A is strictly fat-soluble the question arises as to what the form of combination or dispersion in urine may be. The first possibility to be examined was that vitamin A, although insoluble in water, might be soluble in normal or pathological urine.

The insolubility of vitamin A in normal urine. 2 ml. of halibut-liver oil were added to 100 ml. of normal urine. The mixture was shaken vigorously for $\frac{1}{2}$ hr. and then filtered through four wet Whatman no. 44 filter papers in succession. No vitamin A could be detected in the filtrate. Similarly, vitamin A extracted from pneumonia urines could not be dissolved in normal urines. For example, 40 ml. of a pneumonia urine showing a strong SbCl_3 reaction was extracted with ether and alcohol and the residue, after evaporation, was shaken with 50 ml. of normal urine and filtered through a wet filter paper. No vitamin A could be detected in the filtrate.

The solubility of vitamin A in some pathological urines. Pathological urines were next tested to determine whether they would dissolve vitamin A. In general it was found that urines containing vitamin A were capable of taking up more vitamin when shaken with halibut-liver oil (see Table 2).

Attempts were next made to find how vitamin A is held in solution in urine. Most urines containing vitamin A also contained heat-coagulable protein. No quantitative relation between the amounts of protein and vitamin A excreted could, however, be traced. In one case of nephrosis the daily outputs of globulin, albumin and vitamin A were measured for a month, but no correlation was apparent.

Table 2

Subject	Description	Vitamin A in urine i.u./100 ml.	
		Before shaking with H.-L. oil	After shaking with H.-L. oil
Human	Normal	0	0
"	Pyrexia (unknown origin)	0	18
"	Pneumonia	0	85
"	Pneumonia early	60 (clear)	190 (turbid)
"	Pneumonia	370 (clear)	510 (clear)
"	Proteinuria	68	1650 (turbid)
Dog	Normal	60	225

Note. A solution of 0.5% bile salts, when treated with halibut-liver oil in the same way as these urines, took up 425 i.u. of vitamin A/100 ml.

Precipitation of vitamin A with heat-coagulable protein. When the protein in such a urine was coagulated by heating, all the vitamin A was found in the coagulum. This may be merely an adsorption effect, since when concentrates of the vitamin in the water-soluble state (see below) are heated much of the vitamin escapes precipitation. This suggests that removal of the vitamin by heat coagulation of the protein is only complete when conditions for adsorption are favourable, i.e. much protein and relatively little vitamin A in the solution.

Urines of subjects with pneumonia show the highest vitamin A contents and yet often contain little heat-coagulable protein. In one instance 30 i.u. of vitamin A per 100 ml. were present in such a urine which contained no heat-coagulable protein but gave reactions for proteose and peptone. We have never obtained a preparation containing vitamin A in watery solution which did not give the protein colour reactions.

The concentration from urine of vitamin A in the water-soluble state

The following experiments were performed with a view to devising a method for the concentration of the vitamin.

Precipitation by basic lead acetate. 45 ml. of a urine giving a strongly positive test for vitamin A were treated with 5 ml. of a saturated solution of basic lead acetate and filtered. The filtrate gave a negative test for vitamin A and the residue a strongly positive test. The vitamin A could not be recovered in watery solutions, however, by gassing with H_2S .

Precipitation with acetone. Addition of acetone to urines containing vitamin A yielded precipitates containing no vitamin A. This procedure therefore gave promise of effecting a partial separation of the water-soluble vitamin A complex. Much destruction, however, appeared to occur and this procedure was abandoned.

Adsorption on kaolin. The process of adsorption on kaolin at an acid pH and elution at an alkaline pH has been much used in the purification of enzymes, and its applicability to our problem was tested on the assumption that the vitamin A might be combined as a lipoprotein. A urine containing 19 i.u. of vitamin A/100 ml. was first used. 500 ml. were shaken up with a little kaolin and the pH adjusted to 4.0. The kaolin was filtered off and re-suspended in water, the pH adjusted to 9.0 and the suspension filtered after standing for 2 hr. 71% of the vitamin A was found to have been adsorbed and subsequently eluted and 16% not to have been adsorbed.

This experiment was then repeated with a urine containing 375 i.u./100 ml. Only 19% of the vitamin A was found in the eluate. In a similar experiment using a urine containing 375 i.u./100 ml., 16% was found in the eluate after a first adsorption and 23% of the remainder after a second treatment.

These experiments indicate that while adsorption by this method and subsequent elution is efficient with low concentrations of vitamin A, it is not serviceable for the comparatively high concentrations found in pneumonia.

Adsorption on charcoal. Merck's blood charcoal was used. With urine containing 450 i.u. of vitamin A/100 ml. 33% of the vitamin disappeared after shaking with the charcoal, and of this almost none could be eluted. Thus this procedure is not likely to be of use except for the decolorization of strong preparations obtained by other means.

Concentration in vacuo. Little loss of vitamin A occurred during this procedure, which was often used in making concentrates.

Dialysis. The vitamin A was retained on dialysis through parchment paper against water. Parchment paper sacs permitted rapid removal of salts, the dialysis being first carried out against running tap water and finally against distilled water.

Procedure for concentration of vitamin A in the water-soluble state

Our most effective procedure was as follows. The urine was first fully saturated with ammonium sulphate. The precipitate formed was filtered off on a Büchner funnel, the paper carefully removed and the funnel washed out. The paper was then replaced, and about 100 ml. of distilled water were drawn through the paper. This water was poured repeatedly through the paper till no more of the brown precipitate was removed; the process was repeated with 2-3 changes of water. The extracts were then combined and concentrated *in vacuo* to a volume of 20-50 ml. This concentrate was dialysed in a parchment sac, first against running tap water and later against distilled water, until it no longer gave a precipitate with BaCl_2 and HCl .

Concentrates prepared in this manner were brown and usually contained a little heat-coagulable protein which could be removed by boiling with a drop or two of 5% acetic acid and filtering. The precipitate of heat-coagulable protein contained some vitamin A. The filtrate had the following properties. (1) Vitamin A, a strongly positive SbCl_3 test. (2) Mercuric nitrate test (Millon's reaction for tyrosine): strongly positive. (3) Aldehyde reaction of tryptophan (glyoxylic test): probably positive, but the colour was obscured by the brown colour of the preparation. (4) Arginine reaction (Sakaguchi, with α -naphthol): moderately strongly positive. (5) Biuret reaction: positive, violet. (6) Molisch reaction (for carbohydrate): negative.

From the above findings it appears that vitamin A, as it occurs pathologically in urine, is associated with a non-heat-coagulable protein fraction.

The solution of vitamin A by protein derivatives. In view of the association of vitamin A in the urine with this nitrogenous fraction, it was of interest to find to what extent vitamin A from halibut-liver oil could be obtained in watery solution by means of protein derivatives. A 5% solution of 'Difco' proteose-peptone, salt and halibut-liver oil were ground together in a mortar and filtered twice through wet filter papers. Although only poor emulsification was obtained by this means, the filtrate showed a positive SbCl_3 test of low intensity. These experiments were not carried out quantitatively, but since the halibut-liver oil used contained about 60,000 i.u. of vitamin A/1 ml., only a very small fraction of the vitamin A present in the oil was extracted into the watery phase. A concentration similar to that occurring in the urine in many cases of chronic nephritis was, however, readily obtained.

Concentration of vitamin A per unit of urinary lipid. With all urines containing vitamin A only small amounts of fatty material could be obtained by the

usual procedure of adding KOH and alcohol and extracting with ether, a treatment which would not be expected to cause saponification of triglycerides. When only small quantities were extracted the amounts of fat obtained were too small to be weighed without elaborate precautions to ensure drying to constant weight. Large amounts of urine were, however, collected from a few subjects for the purpose of vitamin D estimations (Table 4). Sufficient lipid to permit accurate weighing was obtained in one instance: the vitamin A concentration was about 23 I.U./mg., which is about 10 times greater than that found in cod-liver oil and would be a reasonable value for human liver fat. The urine examined showed only moderately heavy excretion of the vitamin, and much more concentrated extracts, probably up to 200 I.U./mg., have been obtained in other instances.

Part III. *The vitamin A content of human kidneys*

Experiments with animals have indicated that the kidneys are one of the most important sites of distribution of the relatively small amounts of vitamin A that are not absorbed by the liver. Evidence of the concentration of vitamin A in the human kidney may therefore help in our understanding of the excretion of the vitamin in the urine in disease. The data on specimens obtained at autopsy, given in Table 3, were obtained during an investigation of the vitamin A

Table 3. *The vitamin A content of the kidney at autopsy*

Individual values, in I.U./g., for the vitamin A contents of the liver and kidney were determined at autopsy on subjects dying from accidental death and various groups of diseases.

		Accidental death													
Liver	15	120	150	150	150	150	180	180	300	300	300	375	450	750	750
Kidney	0.6	1.2	0	0.6	1.2	1.2	1.2	1.2	1.2	2.4	6	0.3	2.4	3.6	4.8
		Diseases of alimentary tract													
Liver	6	60	90	90											
Kidney	0	0	0	2.4											
		Organic heart disease													
Liver	22	37	45	45	60	60	60	60	60	90	90	90	120	120	
Kidney	0	0	0	0	0	0	0.6	0.6	1.2	0	0	1.2	0	1.2	
		Respiratory diseases													
Liver	6	12	15	30	37	337									
Kidney	0	0	0	0	0	2.6									
		Nephritis													
Liver	0	1.5	3	3	9	9	9	15	45	60	75	135	375		
Kidney	0	0	0	0	0	0	0	0	0	0.3	0	0.6	6		
		Renal infections													
Liver	15	15	30	37	45	135	135	375							
Kidney	0.6	0.6	0	0	0	0.6	3.6	0.3							

reserves of the liver [Moore, 1937]. It will be seen that in accidental death 14 out of 15 specimens gave positive results in the SbCl_3 test under the particular conditions of concentration adopted. In disease the ranges of the vitamin A reserves of the liver were lower, and the proportion of kidney specimens giving positive SbCl_3 tests was much smaller. Thus in respiratory diseases negative results were obtained for 5 out of 6 specimens, in nephritis for 10 out of 13 specimens.

Part IV. *The excretion of vitamin D in urine*

In view of the frequent association of vitamin D with vitamin A in liver oils experiments were undertaken to find out whether this vitamin can be excreted in urine. Large volumes of urine (in one instance 44 l.) were collected from one normal and three pathological subjects, some of whom were given large doses of calciferol. To extract the fraction which would be expected to include vitamin D, if present, the following procedure was used. The urines were either concentrated by evaporation, or the protein was separated by precipitation. The products so obtained were digested with alkali, alcohol was added and the lipid fractions were extracted with ether. After evaporation the extracts were diluted with arachis oil and dosed to rats which had been kept on Steenbock's diet No. 2965 (yellow maize 76 %, NaCl 1 %, CaCO₃ 3 %, wheat gluten 20 %) until severe rickets had developed.

The unit taken in adjusting the level of dosing was 1 day's excretion by the subject, which was divided into even doses spread over the curative period. In one case the dose corresponded to 6 days' excretion by the subject. The duration of the experiment with the dog, which received 20,000 i.u. of calciferol per day was, however, sufficient to permit dosing at the level of only about half the daily output. The degrees of curing were assessed by X-ray examination, a solution of calciferol being used as a standard. Results are given in Table 4.

Table 4. *The excretion of vitamin D in urine*

Subject	Disease	Approx. daily ex- cretion of vitamin A i.u.	Daily dosage of calciferol i.u.	Total daily excretion of lipoids mg.	No. of days urinary output from which test dose was extracted	Daily ex- cretion of vitamin D i.u.
Human	None	—	9,000	8	1	0
"	Chronic nephritis	350	0	3	1	0
"	Osteomyelitis with nephrosis	800	9,000	35	1	20
"	Nephrosis	30	0	10	6	1
Dog	None	400	20,000	0	$\frac{1}{2}$	0

No vitamin D could be detected in urine from the normal human subject or from the dog, although large doses of calciferol had been given. Negative results were also obtained in the patient with chronic nephritis who received no doses. 20 i.u. of vitamin D were excreted daily by the patient with osteomyelitis who had been dosed with calciferol, and a trace by the patient with nephrosis, who was not dosed.

DISCUSSION

Selectivity of the excretion of vitamin A. Vitamin A is associated in urine with only minute amounts of lipoids and its excretion appears to be highly selective. If we take round figures of 100 i.u./100 ml. for vitamin A, and 600 mg./100 ml. for the lipid content of human plasma, then the concentration of vitamin A related to lipid will be about 0.17 i.u./mg. The exact value will vary considerably in individual cases, both in health and disease, but will remain of the same order of magnitude. In pathological urine the concentration per unit of lipid may be much higher. In the present work an extract containing 23 i.u./mg. of lipid was obtained, and it seems probable that much higher concentrations are frequent. The concentration per unit of lipid in the urine is therefore at least 100 times greater than in blood. The selective nature of the excretion of vitamin A

is further emphasized by the virtual absence of vitamin D from the urines of the human subject and the dog given calciferol.

The conditions determining urinary excretion of vitamin A. A full explanation of the excretion of vitamin A in urine is made difficult by the diversity of the conditions under which it occurs. Thus in the dog heavy excretion is found in normal health. In the human subject vitamin A is absent from the urine in health, but present in some pathological conditions. In the rat we have failed to detect excretion of the vitamin even in disease. No single theory seems capable of explaining all the facts. Even the attractive suggestion that excretion of vitamin A results from injury to the reticulo-endothelial system breaks down in the case of the dog. It is difficult moreover, to understand why, if this system as a whole is involved in the storage of vitamin A, the spleen and bone marrow should not contain large amounts.

Two generalizations, however, seem possible. (1) Urine which contains vitamin A differs from normal urine in its ability to take up more vitamin A, from halibut-liver oil, in a form sufficiently dispersed to pass through damp filter paper. This property appears to be associated with the presence of protein in the urine. It may be noted that Schneider & Weigand [1937, 1, 2] and Hedberg & Lindqvist [1938] have remarked on the tendency of urines containing vitamin A to form emulsions with ether. (2) The two most prominent diseases in which vitamin A is excreted in the urine, chronic nephritis and pneumonia, are among those in which low ranges of reserves of vitamin A have been found in the liver at autopsy [Moore, 1937].

These generalizations clearly do not necessarily hold good in an opposite direction. Urines containing much protein, as in acute nephritis, are often devoid of vitamin A, although they possess the property of taking up the vitamin when shaken with halibut-liver oil. The reserves of vitamin A in the liver at autopsy are sometimes low in diseases in which vitamin A is seldom present in the urine.

Abnormal solubility relationships. The cause of the urinary excretion of vitamin A by pathological human subjects can be pictured most readily as arising from alterations in the relative powers of the liver, blood, kidneys and urine to dissolve or absorb the vitamin. In the normal human subject, as in other vertebrates, vitamin A is preferentially absorbed and retained by the liver to a remarkable degree. The distribution of vitamin A between this organ and the blood stream must be considered as an equilibrium, since in the human subject the vitamin A content of the liver never attains more than some 10% of the concentration which may be assumed, from experiments with many different animals, to represent saturation. In the dog this equilibrium is changed in the direction of increased solution of vitamin A in the blood by the ingestion of alcohol [Clausen *et al.* 1940], but otherwise we know little of the factors controlling the distribution in health.

The relative distributions of the vitamin in the human being in health, pneumonia and chronic nephritis, as far as they can be assessed from data obtained for the various organs and fluids on different groups of subjects, are summarized in Table 5. In both pneumonia and chronic nephritis the amount of vitamin A held by the liver is reduced. In pneumonia the absorptive powers of the blood and kidneys are also reduced and a high concentration of vitamin A passes into the urine, together with abnormal metabolites capable of holding it in solution. In chronic nephritis, the liver reserve is usually more seriously reduced than in pneumonia. The level of vitamin A is often raised in the blood, but lowered in the kidney. The concentration of vitamin A in the urine is usually lower than in pneumonia. These differences may possibly be explained by the

Table 5

	Vitamin A (i.u./100 g.)			
	Liver	Blood serum	Kidneys	Urine
Normal	22,000	70	190	0
Pneumonia	6,300	27	40	190
Chronic nephritis	2,500	120	50	16

Liver values are medians found by Moore [1937]. 'Normals' refer to cases of accidental death. Blood values are means from data of Lindqvist [1938]. Kidney values from means of data given in Table 3. Urine values from means of data given in Table 1.

inefficiency of the kidneys in chronic nephritis, which might lead to the accumulation in the blood of substances which increase the solution of vitamin A.

The occasional occurrence of proteinuria without excretion of vitamin A indicates that all urines capable of dissolving vitamin A do not necessarily contain it. It seems probable that in the human subject at least, functional abnormality of the liver must be involved before excretion of vitamin A takes place.

Quantitative aspects. In pneumonia the amount of vitamin A lost in the urine may exceed the reputed daily requirement of 3000 i.u. Since at a given time the kidneys of a typical healthy adult must contain about 500 i.u. of vitamin A and the blood 2000 i.u. it is clear that excretion cannot be long maintained from these sources without their replenishment from elsewhere. Surprisingly large amounts of vitamin A may be obtained in an invalid diet composed largely of milk, and further amounts might be mobilized during the use of depot fats. In view of the fall of vitamin A in the liver, however, it seems probable that the vitamin A excreted in the urine is derived from this organ. It is important, however, to realize that the daily excretion in pneumonia, say 3000 i.u., is small compared with the liver reserve of 330,000 i.u. typical of the healthy adult. The reduction of the reserve in pneumonia to a median value of 94,000 i.u. cannot all be ascribed to urinary excretion, and much vitamin is presumably lost without leaving any trace of its fate.

In chronic nephritis the daily excretion is usually lower. As in pneumonia, therefore, the reduction of the median value for the liver reserve to 38,000 i.u. cannot be accounted for quantitatively by the urinary excretion.

SUMMARY

1. Vitamin A could not be detected by means of the SbCl_3 test in samples of normal human urine, even from subjects who had received large doses of vitamin A. The reported frequent occurrence of vitamin A in human urine during pregnancy was not confirmed. Carotene was never found in significant amount in urine of any description.

2. The frequent excretion of vitamin A in urine in some human diseases, particularly pneumonia and chronic nephritis, was confirmed. In pneumonia the daily excretion sometimes exceeded 3000 i.u., the reputed daily requirement for this vitamin. The rate of excretion in chronic nephritis was generally less.

3. The presence of large amounts of vitamin A in the urine of the healthy dog was confirmed. Vitamin A was not found in the urine of normal or diseased rats. Not more than traces were detected in the urine of a cat or of normal or diseased rabbits. The attempted blockage of the reticulo-endothelial system by injections of bismuth in the rat or rabbits did not cause excretion of the vitamin.

4. The positive results by the SbCl_3 methods were confirmed in one urine by the presence of the absorption band at $328 \text{ m}\mu$ characteristic of vitamin A, and in two others by biological tests.

5. Some human urines containing vitamin A were clear, others were turbid, but some of these could be filtered to complete clarity without the removal of vitamin A. Protein, not necessarily heat-coagulable, was always present. Urines originally containing vitamin A took up still more vitamin A when shaken with halibut-liver oil. Normal human urine did not do this, but those which contained protein without vitamin A did.

6. Traces of vitamin A were found regularly in kidneys taken at autopsy in cases of accidental death. In disease the amounts present were generally reduced. Under the conditions of the test, negative results were obtained in 5 out of 6 cases of respiratory disease, and in 10 out of 13 cases of nephritis.

7. Vitamin D was absent from, or present only in traces in, the urine of human subjects or the dog, even when large doses of calciferol had been ingested. When 9000 I.U. of calciferol were given daily to a diseased subject with spontaneous excretion of vitamin A, only 20 I.U. daily of vitamin D were excreted in the urine.

8. The excretion of vitamin A in urine is highly selective, the concentration of vitamin per unit of lipid being much greater in urine than in blood. It may be significant that chronic nephritis and pneumonia, the two diseases in which urinary excretion of vitamin A is most frequent, are among those in which very low ranges of reserves have been found in the liver at autopsy. The amount of vitamin A lost in the urine cannot, however, account fully for the diminution of the liver reserves in these diseases.

Our thanks are due to Dr L. J. Harris for his valuable criticism, to Dr B. McArdle for collaboration in the clinical parts of this work, and to the Staff of Addenbrooke's Hospital for permission to investigate the cases under their care.

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