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J Clin Invest. 1933;12(1):87-93. <https://doi.org/10.1172/JCI100497>.

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THE ADDIS SEDIMENT COUNT IN NORMAL CHILDREN

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(Received for publication August 9, 1932)

THE METHOD

In 1925 Addis (1) described a method by which, in a concentrated acid urine, the rate of excretion of protein, casts and red and white cells could be determined. His method, with certain modifications, has been followed here. All of the counts were made on the 12 hour night specimen from 7 or 8 P.M. to 7 or 8 A.M. Addis recommended that fluids be restricted during, and for 12 hours preceding the collection, since in dilute and alkaline urine hyaline casts dissolve and red cells may be completely lysed. With children this rigid restriction of fluid proved impossible. Withholding fluid during the afternoon and night except for 200 cc. at the evening meal, gave urines of such concentration and acidity that they were suitable for a count. Most children had an early supper and collections were started at 7 or 8 P.M. Under these conditions, the urinary pH was between 5.0 and 6.0 and the specific gravity usually well above 1.020. The specimens were treated as described by Addis: "the concentrated night urine is thoroughly mixed by repeated inversion of the rubber-stoppered bottle and a 10 cc. sample is transferred to a special graduated tube, and centrifugalized for five minutes at 1,800 revolutions per minute. The supernatant urine is decanted and pipetted down to a known volume which varies with the amount of sediment as judged by direct observation. The casts and cells are thoroughly mixed in this remaining fluid by means of a capillary pipette and drops are transferred to a hemocytometer. The casts are counted under the low power microscope in several unit volumes of 0.0009 cc., the number depending on the concentration. The cells are counted in unit volumes of 0.0001 cc. with a 1/6 eyepiece of number 10 objective. The calculation is very simple if a slide rule is used. The rates of cast, red blood cell, and white blood and epithelial cell excretion are expressed always as twelve hour rates." For the quantitative protein the method of Shevky and Stafford (2) was followed with a modification in calculation suggested by Addis. In this method the volume of protein precipitated by a solution of phosphotungstic acid in strongly acidified alcohol is determined, the precipitate being packed by centrifugalizing under constant conditions.

161 sediment counts on 74 normal children

Children admitted to the hospital for hernia and eye operations or for observation for various conditions were considered normal when the routine urinalyses were negative. Cases in which there was any possibility of renal irritation were not included. Addis thinks that for cell counts in women, catheterization is essential. In this series no precautions were taken to secure unusual cleanliness, though cases of vaginitis were not included. A number of counts were done on children who were not under hospital care and whose diet and activity, therefore, could be considered more normal. No differences were observed in the counts. The age range was from 4 to 12 years. Children in the lower age groups (4 to 7 years) gave slightly lower figures for protein, casts and cells than the older children, and zero counts were more frequent in this group. The excretion of epithelial and white blood cells was found to be somewhat higher in female than in male children.

The results are shown in Table I; the range and averages are shown in Table II. The series is small and the distribution is so skewed that no statistical analysis has been made. The probable error of the means has been computed. In Table III tentative figures are given marking the upper limit of normal excretion in children from 4 to 12 years of age.

TABLE I

12 hour excretion of protein, casts and cells. 161 observations on 74 normal children 4 to 12 years of age

Protein		Casts		Red blood cells		Epithelial and white blood cells	
Mgm.	Number of observations	Thousands	Number of observations	Thousands	Number of observations	Millions	Number of observations
0	0	0	118	0	108	0	0
1 to 5	8	Over 0 to 2	9	Over 0 to 20	11	Over .0 to .5	133
6 to 10	30	Over 2 to 4	17	Over 20 to 40	12	Over .5 to 1	19
11 to 15	40	Over 4 to 6	9	Over 40 to 60	14	Over 1 to 1.5	4
16 to 20	28	Over 6 to 8	3	Over 60 to 80	11	Over 1.5 to 2	0
21 to 25	13	Over 8 to 10	2	Over 80 to 100	3	Over 2 to 2.5	2
26 to 30	16	Over 10 to 12	2	Over 100 to 120	1	Over 2.5 to 3	3
31 to 35	13	Over 12 to 14	1	Over 120 to 140	1		
36 to 40	8						
41 to 45	4						
46 to 50	1						

TABLE II

12 hour excretion of protein, casts and cells. Range and average in normal children and adults

		Children	Adults (Addis)	Adults (Goldring)
Protein, mgm.	Range Average	3 to 47 18.5±.5		3 to 60 14.6
Casts	Range Average	0 to 12,916 1,085±123	0 to 4,270 1,040	0 to 9,200 647
Red blood cells	Range Average	0 to 129,000 15,181±1,400	0 to 425,000 65,750	0 to 1,530,000 163,000
Epithelial and white blood cells	Range Average	9,000 to 2,822,000 322,184±25,500	32,400 to 1,835,000 322,500	24,000 to 3,400,000 647,500

TABLE III

The upper limit of normal excretion per 12 hours in children from 4 to 12 years of age

Protein.....	35 mgm.
Casts.....	10,000
Red blood cells.....	600,000
Epithelial and white blood cells.....	600,000 (male) 1,000,000 (female)

Comparison with adult normals

Protein. Addis (3) says: "through observations on many normal individuals and on many patients who were passing from what we call Bright's Disease back to 'normality,' we have reached the tentative conclusion that the upper limit of normal variation is in the neighborhood of 30 mgm. of protein per 12 hour period." Our normal range goes somewhat higher than this, i.e., 47 mgm., and Goldring and Wyckoff (4) in 21 adult normals found a range of from 3 to 60 mgm. It is not surprising that our average and normal range is higher than in the adult series. Albuminuria in healthy children is a common finding from the age of six to puberty and is very common during puberty.

Casts. As in the adult series of Addis, practically all of the casts were hyaline. The upper normal limit is higher than that given by either Addis or Goldring, but the average is the same. Thirty-eight of 161 cast counts were above the average, while 118 or 73 per cent were zero counts. In Addis's series there were 39 of 74 cast counts above average and 40 per cent zero counts. Possibly the high percentage of zero counts in the children of this series is due to the fact that fluids were not rigidly restricted.

Red blood cells. The presence of red cells in the urine is frequently regarded as indicative of some abnormality in the urinary tract. The work of Addis, and others has shown that normal adults may excrete

small numbers of red cells in the urine. That this also holds for normal children is evident from the studies presented here. Red cells are not constant urinary constituents. In some children they have been found at one examination and not at another, and, in others, repeated examinations have failed to disclose their presence.

Our average (15,181) is lower than that of Addis (65,750), and our percentage of zero counts (67 per cent) is higher than that of Addis (51 per cent).

Several factors may account for the lower red cell excretion in children. It is conceivably the result of failure to restrict fluids rigidly. It may be related to difference in kidney weight. While the child has the same number of glomeruli and tubules as the adult, it is obvious that they must be smaller. Addis has found that the actual red cell excretion may be obscured at times when the salt concentration in the urine becomes so low that red cells are invisible. Children, and especially hospital children, have a very low salt intake. Three of our normal children who had been showing zero red cell counts were on two occasions given 5 grams of salt at the evening meal. In all of these the red cell counts remained zero. On the whole it seems fair to assume that the relatively simple life of the child, in which stresses, strains and complicating features (latent infection, local disease of the urinary tract) are correspondingly few, is a factor of some importance in determining the lower erythrocyte excretion.

White blood cells and epithelial cells. The average for children is practically the same as that for adults. The high range and average in this series is probably due to the fact that we did not catheterize female children. The average for male children is about half that of the adult group.

The sediment count compared with routine urinalysis

The results of 51 sediment counts have been compared with the reports from the routine microscopic examination of the same specimen. Ten cc. of the thoroughly mixed urine were used for both procedures. The specimen for routine examination was centrifuged for the same length of time and at the same rate of speed as in the quantitative method. The only difference was that in the routine examination the sediment was examined practically undiluted, while in the count the sediment was always mixed in from 1 to 3 cc. of urine. More than the usual time and care were taken for the routine examination. Cases were chosen where the volume was approximately the same (about 200 cc.) and where the changes were slight or moderate. They were cases of acute infection, subsiding nephritis and rheumatic carditis.

Protein. Table IV indicates a relatively close agreement between quantitative protein and qualitative albumin reports. With percentage values up to .01 per cent, the qualitative tests were reported "negative"

or "trace," and with urine volumes of around 200 cc., the amounts excreted were within normal limits.

TABLE IV
Comparison of quantitative protein and routine qualitative albumin

Qualitative	Quantitative per cent	Volumes up to 200 cc. mgm.
Negative or very faint trace.....	.007	14
Trace to heavy trace.....	.01	20
Heavy trace to +.....	.01 to .05	20 to 100
+ to +++.....	.05 to .1	100 to 200
++ to ++++.....	.1	200

Casts. With cast excretion from 6,000 to 79,000 in 12 hours, the qualitative report was "negative" or, in a few instances, "1 to 2 per high power field." With counts of 122,000 to one million, most of the qualitative reports were "1 to 2 per H. P. F." In one case where the count was six million the routine report was "10 to 20 per H. P. F.," while in two other cases where the counts were twelve and fifteen million respectively the routine report was "5 to 10 per H. P. F." Such discrepancies were not common. Four times out of 51 tests in which the cast counts were zero, the routine examination showed a few casts.

Red blood cells. Among 18 counts which ranged from 222,000 to 2.4 million red cells in 12 hours, there were 11 routine reports of "1 to 10 per H. P. F." and 7 of "no red blood cells." When the red cell excretion was from 2.5 to 82 million in 12 hours, the routine reports showed "1 to 20 red cells per H. P. F." As the red cell excretion increased through this range, there was little or no difference in the routine reports. With excretions of 189 to 571 million the routine analysis yielded "5 to 60 red cells per H. P. F." In cases of microscopic hematuria it cannot be said that these methods showed a very close agreement.

Epithelial and white blood cells. With white cell excretion under one million most of the routine reports were "3 to 5 per H. P. F." with an occasional "zero" report and rarely "10 to 20 per H. P. F." When the excretion was from 1 to 10 million most of the reports were "5 to 10 per H. P. F." With excretion from 10 to 75 million the common report was "10 to 20 per H. P. F.," and notes mentioning the presence of "clumped white cells" were frequent.

A summary of the comparative results yielded by the two techniques indicates that the major discrepancy occurs in estimating red cells. It is likely that this is due in part to the difficulty which even practiced workers experience in recognizing red cells and in part to the fact that books and clinical pathologists have long insisted that the presence of red cells in the urine means some abnormality in the urinary tract. The physician, therefore, shrinks from reporting erythrocyturia in an individual who has no nephritis, papilloma, or renal tuberculosis to account for it. In

discussing this matter with laboratory workers, many have admitted that they seldom report an occasional red blood cell in the urine because of its supposedly grave import. Traces of albumin and a few white blood cells are commonly reported in well children, and the pediatrician does not become alarmed because of their presence. Similarly there is need of a tolerant attitude toward occasional red cells in the urine and such tolerance need not decrease our respect for their significance when they are present in increased numbers.

DISCUSSION

The wisdom of introducing any new method of examination which is more time-consuming than the old is questionable unless it can be shown that the character of the information yielded as well as the uses to which it can be put are improved by the new routine. The advantages of quantitative data over qualitative data in general do not call for discussion. It is essential only to determine whether quantitative urine studies justify in added usefulness the time necessary to procure them. A completely satisfactory answer to this question, insofar as it involves the extent to which one method should replace the other, is as yet impossible as the full sphere of usefulness of sediment counts is not yet known. At present it would seem that the method should not replace the routine urinalysis. Experience, however, has indicated that many cases exist in which the information yielded justifies, from the standpoints of both therapy and prognosis, the added expenditure of time. In this clinic the method has proved valuable in studying patients with nephritis, not only in providing quantitative and therefore comparable data for following the course of disease, but also in permitting early recognition of the effect on the kidneys of pharmacologic procedures or intercurrent infection.

It has been contended that the method is only approximately accurate. The truth of this contention was recognized by Addis (5) who pointed out that it is unnecessary to make the method quantitative in the strict sense of the word. "At least for clinical purposes, we want to know the rates in terms of orders of magnitude. It is immaterial whether the urine contains one million or one million one hundred thousand casts, although, it is essential to be certain whether there are thousands, tens of thousands, hundreds of thousands or millions." Wide variations are therefore to be expected in normal subjects, and a close division between normal and abnormal is impossible. With children the range of variation is further increased by variations in urine volume resulting from inability to enforce rigid fluid restriction. With water restricted for 24 hours Addis succeeded in obtaining with adults fairly constant 12 hour night collections of around 380 cc. With children especially, therefore, it is essential to hold a rather broad conception of what may come within the range of normal. The figures presented in this paper must be regarded as very

roughly approximate only, although the attempt was consciously made to err on the side of including too much, rather than too little, in the normal range.

SUMMARY

The technique of Addis for the quantitative determination of the excretion of protein, casts and cells in the urine has been applied to normal children, aged 4 to 12 years. The data have been compared with the findings on routine urinalyses and with the results by the Addis technique in adults. A tentative standard has been set up for normal children between the ages of 4 and 12 years.

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