Results: The normal rats used had an oxygen consumption varying from 1420 to 1670 mg O_2 per kg per hour, but for the individual rat the figure was constant within a limit of 8%. The O_2 consumption of thyroidectomized rats was 900 to 1200 mg per hour per kg. Medication with varying doses of thyroxine in micrograms per 10 g weight and given daily for 3 days, and corresponding medication with thyroid globulin, calculated on thyroxine content, gave the results presented in Tables I-III.

While it seems that in normal rats the dose of 25 γ thyroxine might fall within the range of best measurable response, the spread of figures is too wide for acceptable conclusions to be drawn. Thyroidectomized rats are about 30 times more sensitive. Although the number of experiments presented so far is limited, a tentative interpretation of results seems to be permissible. They indicate that the percentage metabolic increase plotted as a function of dosage follows a logarithmic curve. A minimum dose is required before any response obtains. Increase of dosage produces initially a rapid increment of metabolism, which approaches a more gradual linear function at 20 to 35% increase and finally becomes slower. For comparative measurements, an increase between 25 and 30% seems to be the most favorable range. Surmising that thyroxine is the sole stimulator of metabolism in the thyroid globulin, between 0.19 and 0.25 γ thyroxine given in this form appears to be as active as 0.75 γ crystalline thyroxine, which would mean that thyroxine in natural linkage is 3 to 4 times more potent than the racemic substance.

10037

Hemolysin in the Urine in Aplastic Anemia.

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Ample evidence is at hand to prove that a substance which is lytic for erythrocytes is present in the urine of human beings under normal conditions. Few studies are available of this phenomenon in patients with disorders of the blood in which hemolysis might be a factor. Accordingly, the urines of patients with certain representative types of blood dyscrasia have been investigated for their

content of lysin. In the urine of patients with aplastic anemia lysin has been found to be present in a form which is different from that described for any normal or any pathologic state studied hitherto.

McKee[1] first observed the lytic activity of human urine. Ponder[2] found lysin in more than 90% of normal human urines and states that although it may be absent on any one day it appears regularly in all normal individuals. Abels[3] in a detailed study of the subject examined 2,026 normal urines and found lysin in 93%. No individual secreted non-lytic urine for more than 4 days in succession. The urines from 72 individuals were examined for periods of 2 to 5 weeks and those of 400 individuals for 1 day only. The lysin was heat stable, alcohol soluble, diffusible, adsorbable, destroyed finally by alkali, and inhibited by plasma proteins, lecithin and cholesterol.

For the simple determination of lytic effect, human erythrocytes were washed 3 times in 5 volumes of isotonic saline and made up in a concentration of 1% in saline. Urine samples measuring 0.5, 0.25 and 0.125 cc were measured and mixed with 1 cc of the suspension of erythrocytes. The tubes were kept at 37° in a bacteriological incubator for 4 hours. The titre of the urine lysin is indicated by the lowest dilution of urine which effects complete lysis of erythrocytes in that time.

For the recovery of lysin from the non-lytic urine of cases of aplastic anemia, 1 volume of urine was brought to pH 1 with 6 N HCl. This was refluxed for 30 minutes, cooled, brought to pH 6 with 2 N NaOH and evaporated to dryness on the water bath. The residue was ground with 1 volume of 95% alcohol for 15 minutes and filtered. The precipitate was washed twice with one-fourth volume of 95% alcohol with repeated grinding and the washings were added to the filtrate. The whole was evaporated to dryness on a boiling water bath. The residue was taken up in one-half volume of isotonic saline filtered and adjusted to a pH of 5.6. Of this final saline solution 0.5 cc was added to 1 cc of 1% suspension of washed human erythrocytes and placed in an incubator at a temperature of 37°C. The tubes were examined, without removing them from the incubator, every 5 minutes for 15 minutes and at intervals of 10 minutes thereafter.

All of the 8 cases of aplastic anemia tested were typical. In 5 the diagnosis had been confirmed by examination of the bone marrow. In all cases repeated transfusions of blood as well as heavy treat-

[1] McKee, C. S., *Brit. Med. J.*, 1915, **2**, 596.

[2] Ponder, E., *Brit. J. Exp. Path.*, 1921, **2**, 34, 192.

[3] Abels, J. C., *Am. J. Physiol.*, 1934, **107**, 603.

ment with liver extract and with iron failed to cause improvement. All these patients had been ill with anemia for months or years, and showed little or no evidence of regeneration in the peripheral blood.

Table I shows the results of testing normal urine for the presence of lysin. Of 2,460 tests, 2,234 were lytic in the experiments of Ponder and of Abels. In Table II are shown the results of testing 89 specimens from 8 patients with aplastic anemia. In no instance was any lytic activity present on direct test even though the observations were continued over weeks. It has been previously noted that normal urines of pH higher than 6.2 were almost invariably non-lytic unless extremely ammoniacal. In the present study of 89

TABLE I.

Controls from average population	Period of observation	Lytic specimens	
		No.	%
2,460	1 day to 4 months	2,234	91

TABLE II.
Hemolysin Before Hydrolysis in Urine of Patients with Aplastic Anemia.

Patients	No. of specimens	No. of days	No. lytic
M.J.	22	18	0
A.C.	21	17	0
D.L.	19	16	0
G.M.	14	12	0
M.R.	7	7	0
C.D.	5	5	0
L.C.	2	2	0
C.J.	2	2	0
Total	92		0

non-lytic specimens, only 4 were of a pH greater than 6.2. Thus this finding was completely contrary to any result obtained in normals and warranted further study. We felt, therefore, that if the lysin were excreted it must be in some bound or conjugated form; and that if mildly hydrolyzed at pH 1 it would again be demonstrable. We had previousy noted[3] that the lysin in the urine could withstand such acid treatment with unimpaired activity.

Accordingly, the non-lytic urines were refluxed for 1 hour at 100°C at pH 1, neutralized to 5.6, evaporated to dryness, and the residue extracted with alcohol, since it was known that the lysin of the normal urine is soluble in alcohol. As controls, 4 normal urines which were known to be lytic were subjected to the same procedure and also 4 normal urines which had been found to be non-lytic. The results are shown in Table III. Of the urines of patients with

TABLE III.
Hemolysin After Hydrolysis in Urine of Patients with Aplastic Anemia.

Patients	No. of specimens	No. of days	No. lytic	Time required for lysis
				min
J.J.	8	8	6	10-10-15-30-30-30
A.C.	4	4	3	5-15-15
D.L.	8	8	8	5-5-20-30-30-40-40-40
G.M.	4	4	4	5-20-20-20
M.R.	5	5	4	10-15-20-45
C.D.	5	5	5	15-20-25-40-40
L.C.	2	2	2	25-35
C.S.	2	2	2	15-15
Total	38		36—94%	
Normals				
J.A. (lytic)	4	4	4	
J.A. (non-lytic)	4	4	0	

aplastic anemia that were tested, none were lytic before hydrolysis, but after hydrolysis 88% were productive of lysis. In the control tests, on the other hand, no lysin was present after hydrolysis in the normally non-lytic urines, though it was still present after hydrolysis in the normally lytic urines.

As further controls, urines were examined from patients with pernicious anemia, Hodgkin's disease, and microcytic anemia due to lack of iron. The results are shown in Table IV. Over a period

TABLE IV.
Hemolysin in Urine of Patients with Blood Dyscrasias Other Than Aplastic Anemia.

Cases	No. of specimens	No. of days	No. lytic
Pernicious anemia	16	14	14
" "	20	16	19
Microcytic "	16	14	14
Hodgkin's disease	10	10	8

of 54 days, 62 urines were examined, and of these 55 showed lysis—approximately the expected result in normals.

From the results it is clear that a lysin which is present in the untreated urine of normal individuals, of patients with pernicious anemia, and of patients with microcytic anemia, is not demonstrable in the untreated urine of patients with aplastic anemia. By mild acid hydrolysis the lytic property may be restored in the urine of patients with aplastic anemia. This is an abnormal finding, however, since no lysis is caused by normally non-lytic urine from normals or from patients with other disease states after acid hydrolysis. Suitable controls establish the fact that the lysis is not due to pH, to tonicity, or to the ordinary physical factors productive of break-

down of erythrocytes. It seems probable that the normal lysin is present in the urine of aplastic anemia but is excreted in a bound form. Studies of Ponder[4] and of Ponder and Abels[5] suggest that the action of the ordinary inhibitors of lysis is a physical one on the erythrocyte and not a combination of inhibitor with lysin which renders the lysin inert.

Because of the relatively weak hydrolysis required to free lysin from the bound form in urines from patients with aplastic anemia, it is reasonable to suspect that some loose linkage, such as a glucuronate, is present rather than some more permanent conjugation.

Further studies of the nature of the lysin and of the method of conjugation are in progress.

Summary and Conclusions. 1. The urine of patients with aplastic anemia contains no lysin for erythrocytes when tested directly. 2. Lysin appears after hydrolysis at pH 1 and 100°C for 1 hour. 3. Such hydrolysis does not result in the production of lysin in urines which are non-lytic normally.

10038 P

The Comb of the Baby Chick as a Test for the Male Sex Hormone.

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This work is a continuation of that already reported[1] on the use of comb growth in white leghorn chicks for the assay of male sex hormone activity. The method previously employed involved the daily application of 0.1 cc of sesame oil, containing crystalline androsterone, to the region of the comb; the chicks were started on the 6th day after birth, treatment was continued for 10 days, and the combs were excised and weighed on the 17th day; the total dosage varied from 0 to 500 gamma, which corresponds to 0-50 gamma per daily application. In all of these experiments, there

4 Ponder, E., *Proc. Roy. Soc.*, 1925, **98**, 484.

5 Ponder, E., and Abels, J. C., in press.

*Hiram N. Vineberg Research Fellow in Gynecology.

1 Frank, R. T., and Klempner, E., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 763.