of the photoelectric colorimeter of red cell volume of rat blood on a single drop of blood obtained from the tail vein. Hemoglobin content may be determined on the same sample.

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Specific Substances in the Urine of Leucemia Patients.

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Miller, Wearn and Heinle[1] reported specific proliferations of cells in the organs of guinea pigs which had been given daily injections of extracts of urine from patients with chronic myeloid and lymphoid leucemias. In the course of further experiments with the same extracts attempts were made to further concentrate the substance. This was accomplished by reducing the pH of the final water extract to 1.5 or 2.0 with hydrochloric acid and shaking vigorously for 15 minutes with an equal amount of chloroform. The chloroform and water were separated and the chloroform removed by vacuum. The residue was resuspended in alkaline water at a pH of 7.4 to 7.6. Each cc of the extract was computed to equal from 1000 to 1500 cc of urine. These extracts were given to guinea pigs in step doses starting with ¼ cc and increasing every 5 days until 1½ or 2 cc was given daily. Animals on such regimes again presented specific pictures in 4 to 6 weeks.

Because we felt that neither of the preceding methods of extraction seemed to yield as much active material as was present another method has been evolved, and for this we have adapted a modification of McCullagh's method of extracting androgens from urine. The urines are hydrolyzed by boiling at a pH of 2.0. It has been found that such a pH in most instances could be obtained by the addition of 40 to 45 cc of concentrated hydrochloric acid to each 1000 cc of urine. After cooling 100 cc of chloroform is added to each 1000 cc of urine and this is stirred violently with an electric stirrer

[1] Miller, F. R., Wearn, J. T., and Heinle, R. W., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 478.

for 20 minutes. The chloroform is then separated and the process repeated 5 times. The separate parts of chloroform are added together and if an emulsion is present this is broken by the addition of a few cc of 10% sodium taurocholate solution. After filtering the chloroform is then distilled off leaving a brown black greasy substance. This substance is then emulsified in water by the addition of a small amount of 5% sodium hydroxide. The emulsion is brought to a pH of 7.4 to 7.6 and after it is suspended in this manner it is boiled for 20 minutes and is ready for use. Each cc of the emulsion is equal to 60 cc of urine. This extract is obtained from a smaller amount of urine than the final solution made by the second method, however each cc contains more solid material.

Such extracts of urine from patients with chronic myeloid leucemia were given in step doses to 4 guinea pigs. One 1-eighth cc was used as the initial daily dose and every 5 days the dose was increased until a dose of 1½ or 2 cc daily had been reached. Within 4 to 6 weeks the animals had lost weight, were in ill health so that they were either sacrificed or died. Each of these animals presented myeloid proliferations in liver, spleen, adrenal, lung and kidney similar to those mentioned in the earlier report. Two of these animals had spleens which weighed 3.5 g.

Similar extracts of urine from one patient with acute lymphoblastic leucemia and the extracts of urine from one patient with chronic lymphoid leucemia were given to 2 pairs of guinea pigs. One of each pair of these guinea pigs died in 2 weeks on doses similar to those given the extract from urine of patients with chronic myeloid leucemia. In these there was marked lymphadenopathy as well as lymphocytic proliferation in the liver, spleen and adrenal. The animal which received the material from the acute leucemia exhibited amaturation of the bone marrow. The other 2 animals died in 5 weeks with findings similar to the first 2 but these were not so marked.

Extracts of urine of 2 normal individuals gave no specific cellular response.

Conclusions. By a modification of McCullagh's[2] method for extracting androgen from urine we have been able to improve the method of extracting specific substances from the urine of leucemia patients. These substances give specific cellular reactions in guinea pigs. We are now testing saponifiable and non-saponifiable fractions of the chloroform residue which have been prepared by Hause.

2 McCullagh, D. R., and McLin, T. R., *Endocrinology*, 1938, **22**, 120.