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Specific Substances in the Urine of Leucemia Patients.

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In previous reports^{1, 2} we have shown that extracts of the urine of patients with leucemia contained substances capable of inciting cellular proliferation in the organs of guinea pigs similar to those found in human leucemias. In one of these reports² it was shown that the substances obtained were specific for the type of leucemia from which the urinary extracts were made.

It has seemed obvious that the chloroform extracts described in the earlier communication contained many inert materials as well as the active principles. Because of this we decided to fractionate the chloroform extracts and tried to trace the active principles preparatory to chemical analysis.

The following method has been developed to fractionate the extracts of urine of patients with chronic myeloid and chronic lymphoid leucemia. It applies particularly to the fractionation of the former.

The urine is extracted with chloroform as before.² The chloroform is removed by distillation and the residue is suspended in 200 to 300 cc of 10% sodium hydroxide. This suspension is extracted 8 to 10 times with 100 cc portions of ethyl ether to remove any substances which will not react with sodium hydroxide. The fraction soluble in ethyl ether (A) is recovered by removal of the solvent. The residual alkaline solution (B) is reacidified with 30 to 50 cc of concentrated hydrochloric acid. The acid solution is extracted with chloroform, the extract after removal of the chloroform is extracted with successive portions of petroleum ether (C); these extracts are combined, distilled *in vacuo*. The residue crystallizes readily in the cold.

Various quantities of urine have been handled in this manner; 30 to 50 liters yield from 3 to 6 g of the petroleum ether-soluble fraction.

The 3 fractions of the extract from the urine of patients with chronic myeloid leucemia have been injected into guinea pigs. Four animals given the alkali-soluble fraction (B) exhibited the same

¹ Miller, F. R., Wearn, J. T., and Heinle, R. W., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **41**, 479.

² Miller, F. R., and Hause, W. A., *PROC. SOC. EXP. BIOL. AND MED.*, 1940, **45**, 387.

changes that we have reported previously. Three animals received the ethyl ether-soluble fraction (A) and although they became ill they showed no proliferation of myeloid cells. Five guinea pigs were given the petroleum ether-soluble fraction (C) in 3 or 4 doses of $\frac{1}{2}$ cc each (50 to 140 mg per dose in olive oil) on successive days and the animals were observed until they showed signs of being ill. Autopsies were performed 3 to 4 weeks after the first injection and proliferation of myeloid cells in the liver, lungs, spleen and adrenals was greater in extent than in any of our previous animals. Three of these animals had white blood cell counts of 40,000 the day of autopsy. The blood smears contained 10 to 20% myelocytes, a few myeloblasts and low percentages of lymphocytes.

Proliferation of lymphoid cells occurred after the use of each of the 3 similar fractions of the extracts obtained from the urine of patients with chronic lymphoid leucemia with no apparent variation. This method, therefore, does not aid in tracing the active principle of this extract.

For control experiments 36 liters of urine of normal individuals were fractionated in the same manner. The yield of the final petroleum ether-soluble fraction was less than one gram. This material produced no specific cellular response.

A method is presented for partially purifying the active principle found in chloroform extracts from the urine of patients with chronic myeloid leucemia. This method does not apply to similar extracts from the urine of patients with chronic lymphoid leucemia.

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Percutaneous Application of Sulfanilamide in Animals and Men.

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As has been described,¹ it is possible to obtain a chemotherapeutic effect in animals and men by the percutaneous application of external disinfectants. In this respect special attention was given to p-chloro-

¹ Zondek, B., in press.