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A Characterization of a Urinary Fraction Capable of Producing Myeloid Metaplasia in Guinea Pigs*

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Heinle, Wearn, Weir, and Rose[1] reported myeloid hyperplasia and metaplasia in guinea pigs following the injection of extracts and adsorbates of human urine. The majority of potent preparations had been obtained from patients with chronic myeloid leukemia. The highest yield of active material appeared to result if the urine was processed by the benzoic acid adsorption method of Katzman and Doisy.[2] We have now further purified this fraction and studied some of its chemical and physical properties as well as some of the quantitative aspects of its effects on guinea pigs.

The benzoic acid adsorbate, prepared from urine of patients with chronic myeloid leukemia, was freed of adsorbent by extraction with acetone. The insoluble residue was leached with a sufficient volume of M/2 boric acid buffer (pH 8.5) to produce an extract of a pH greater than 7. The solution was acidified with acetic acid (pH 4.3) and treated with 1.25 volumes of acetone in the cold. The resulting precipitate was separated and dissolved for assay purposes in saline solution containing phosphate buffer (final pH 7.5). Guinea pigs were injected daily for 8 days with equal doses of this preparation, sacrificed on the 9th day and examined histologically as previously described.[1] The same lesions were again observed but at dose levels usually well below those hitherto required. The results obtained with one of our most potent preparations (1H) are listed in Table I. They demonstrate a correlation between dose and intensity of biological response adequate for testing the stability of this product. Only part of its activity was destroyed at pH 7.5 upon heating on steam bath for one hour. Partial inactivation also occurred upon exposure to pH 1.1 at 27.5° for 24 hours while a solution adjusted to pH 11.2 lost most of its potency at 27.5° in 24 hours. An active solution enclosed within a Visking membrane, retained its potency upon dialysis against distilled water (66 hours at 3°). Our products contained nitrogen but only a trace of phosphorus (Preparation 1H after dialysis 12.5% N, 0.1% P). They developed an intense pink color with carbazole and sulfuric acid,[3] indicating the presence of substantial amounts of carbohydrate. Most of the nitrogen could be accounted for colorimetrically as biuret nitrogen. Treatment with trypsin (Fairchild) resulted in an increase in nitrogen not precipitable with trichloracetic acid and a loss of about ⅘ of biological activity. Although preparation 1H migrated in a single boundary in the electric field at the rate of -5.30×10^{-5} cm^2 $volt^{-1}$ sec^{-1} (Tiselius apparatus; veronal buffer pH 8.53, $\mu = 0.1$, 0.5°) we cannot regard this product as homogeneous. The

TABLE I.
Biological Assay of Preparation 1H.

Urine equiv. of total dose, cc	Total No. of animals	No. of animals, with involvement of organs*				
		0	1	2	3	4
50	6	5	1			
100	5		3	2		
200	3			3		
250	2			1	1	
500	9			3	6	
1000	3			1	1	1
2000	3					3

* 1 organ = spleen, 2 organs = spleen and marrow, 3 = spleen, marrow, and liver or adrenal, 4 = spleen, marrow, liver, and adrenal.

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2 Katzman, P. A., and Doisy, E. A., *J. Biol. Chem.*, 1932, **98**, 739.

3 Gurin, S., and Hood, D. B., *J. Biol. Chem.*, 1939, **131**, 211.

spectral characteristics ($E_{420} = 0.203$, $E_{440} = 0.199$, $E_{520} = 0.286$, $E_{540} = 0.320$ per mg; cell length 1 cm) of the color produced in the carbazole reaction do not agree with those of any simple mixture of monosaccharides. Furthermore, after 0.7 saturation with sodium sulfate, the liquid phase contained significantly more activity and carbohydrate per mg of nitrogen than the precipitate.

Our data suggest that the active principle of our preparations is a protein or glycoprotein. It differs in its chemical and physical properties from the active preparation with similar or identical biological effects obtained from leukemic urines by Turner and Miller.[4] The possibility still exists that their product which is regarded as a ketoacid occurs as a prosthetic group in our preparations. However, the loss of activity which we observed on treatment with trypsin would indicate that any prosthetic group present ought to be less potent than the intact molecule.

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A Comparison of Various Methods of Demonstrating Influenza Virus in Throat Washings.*

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The detection of influenza virus in throat washings is an important method, during epidemics, of determining the causative virus as rapidly as possible. Methods of isolating influenza virus in ferrets and mice,[1] in hamsters,[2] and in chick embryos by amniotic[3] or allantoic[4] inoculation have been described, but a comparison of the efficiency of these 4 methods on the same group of throat washings has not been available. During the epidemic of influenza A in 1943-44, 83 throat washings from military personnel[5] were tested in ferrets or hamsters, or in both species, and a smaller number of the same washings were inoculated into chick embryos.

Methods. Ferrets were inoculated intra-

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