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# Specific Stimulators of Hematopoiesis from Beef Liver.

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In recent papers, we described the biological action,[1] and some of the chemical properties[2] of two substances found in the urine of patients with various diseases of the leucemia group; fractions from urine containing these substances, when injected into guinea pigs, produced specific myeloid or lymphoid proliferation in the organs of the animals. A theory was proposed to explain the biological significance of the two substances;[1] this theory included the assumption that the two specific materials were products of normal metabolism. We have tested this assumption by investigating normal beef liver as a source of the two substances.

The present work was designed primarily to determine whether the two stimulating factors occurred in normal organs. For this reason, the extraction method that proved successful in the case of urine[2] was used again. The separation of the active material from impurities was not quite as complete as with the urine extracts; thus, the lead salts soluble in ether retained activity, as shown in Table I. It was possible, however, to demonstrate the presence of a mixture of the two stimulating factors in the lipids of beef liver. Such a mixture, when injected into guinea pigs, gave a biological response resembling Hodgkin's disease.[1,2] The mixture was separated into two fractions producing proliferation of either the myeloid or lymphoid cells respectively; the two fractions were hydroxy-acids (lymphoid) and non-carbinol acids (myeloid).[2] This separation is illustrated by the results shown in Table II.

The fraction from urine that stimulated the proliferation of myeloid cells was converted previously[2] by catalytic hydrogenation to material that stimulated lymphoid proliferation; the reverse change was accomplished by oxidation. These changes have been effected with similar fractions from beef liver (Table II). The similarity in chemical properties illustrated by these reactions indicates that the factors of urine and liver that produce myeloid proliferation are identical; this applies also to the factors producing lymphoid proliferation. The major result of this work lies in the acquisition of an unlimited source of material for further study. The isolation of the stimulators in pure form from the urine of patients with leucemia seems difficult or impossible because of the limited supply of urine available.

*Separation of Active Fractions from Beef Liver.* Fresh beef liver was ground and dried; the dry material was thoroughly extracted with ethanol. The residue remaining after the distillation of the ethanol was leached with water until the water extracts were colorless. A portion of the residue, weighing 2 kg and corresponding to 43 kg of liver, was dissolved in 3 l. 95% ethanol. A solution of 600 g potassium hydroxide in 3 l. 95% ethanol was added. The mixture was heated to reflux for 4 hours. It was diluted with 3 l. ice water and extracted with petroleum ether (b.p. 30-60 degrees); this removed the neutral material soluble in petroleum ether. The aqueous solution was acidified with hydrochloric acid and the mixture was extracted with petroleum ether to obtain a fraction containing acid and phenolic material. The residue (A) from the evaporation of the petroleum ether was purified further by the same methods that proved effective with urine extracts,[2] except that the partition between petroleum ether and aqueous methanol[2] was omitted. The letters used in the previous paper[2] are used again in Table II to denote similar fractions. Fraction B from beef liver was esterified with a solution of dry hydrogen chloride in methanol;[3] after

1 Miller, F. R., and Turner, D. L., *Am. J. Med. Sci.*, 1943, **206**, 146.

2 Turner, D. L., and Miller, F. R., *J. Biol. Chem.*, 1943, **147**, 573.

3 Pechmann, H., *Ann. der Chem.*, 1891, **261**, 159.

TABLE I.
Experiments with Discarded Fractions from the Purification.

| Material | No. of animals | Dose, g | Dose as crude liver (calculated) | Biological reaction |
|---|---|---|---|---|
| Acids forming ether-soluble | 3 | 0.6-1.4 | 220-510 g | 0 |
| lead salts | 3 | 2.4-3.0 | 1000-1300 g | 0 to (++) H |
| Material from aqueous filtrate of lead salts | 4 | 0.2-0.4 | 15-20 kg | 0 |
| Neutral fraction | 2 | 2.5 | 1200 g | 0 |

TABLE II.
Separation and Interconversion of the Two Active Substances.

| Material | No. of animals | Dose, g | Dose as crude liver, g (calculated) | Biological* reaction |
|---|---|---|---|---|
| B (acids) | 6 | 0.6-1-8 | 260-780 | (++) to (+++) H |
| C (hydroxy-acids) | 6 | 0.09-0.16 | 740-1200 | (+) to (+++) L |
| D (non-carbinol acids as methyl esters | 5 | 1.1-3.5 | 650-2000 | (++) to (+++) M |
| E (reduced D) | 5 | 0.8-1.8 | 400-900 | (+) to (+++) L |
| F (oxidized C) | 6 | 0.1-0.2 | 560-1100 | (+) to (+++) M |

* H represents lesions in the animals that resemble the lesions of Hodgkin's disease; M and L similarly represent lesions of the myeloid and lymphoid type respectively.

acidic material had been removed from the product by extraction of its ethereal solution with sodium carbonate solution, the esters were separated into carbinols and non-carbinols in the usual manner.[4]

*Interconversion of Active Fractions from Beef Liver.* The non-carbinol esters (D) were hydrogenated in ether using Adams catalyst. This procedure followed that used for the corresponding fraction from urine;[2] the product was designated "E."

Hydroxy-acids (C) were oxidized with permanganate as described previously.[2] This product was designated "F."

*Administration of the Fractions to Animals.* Guinea pigs were given the material in 3-4 equal doses at 4-day intervals. The total dose is shown in the accompanying tables. In the tables, the numbers in the column representing the total dose in terms of fresh liver were calculated by assuming that the total weight of each fraction represented the quantity of raw material used in the preparation. Possible losses of active material in the fractionation, and the possibility that the active material was distributed through the fractions without a sharp separation should be considered in evaluating the significance of these numbers.

The material was injected subcutaneously or intramuscularly. The larger doses were given undiluted; the smaller in sesame oil. The guinea pigs were killed when they became moribund; this occurred 3 to 6 weeks after the first dose was given; sections of lungs, liver, spleen, lymph nodes, kidney, adrenal, and bone marrow were taken for histological examination.

The details of the experiments are summarized in the accompanying tables.

*Nature of the Induced Lesions.* The lesions produced in the guinea pigs given material B were the same as those described elsewhere;[1] the latter occurred in guinea pigs given a similar fraction from the urine of patients with Hodgkin's disease or monocytic leucemia. These lesions are identified by the letter "H" in the tables; the purely myeloid or lymphoid lesions are designated "M" and "L" respectively; they have been described previously.[1] The intensity of the biological response is roughly indicated by the repetition of (+) signs.[2]

*Summary.* The two substances that stimu-

[4] Marker, R. E., *J. Am. Chem. Soc.*, 1938, **60**, 2442.

late hematopoiesis, previously found in the urine of patients with diseases of the leucemia group,[1,2] have been shown to occur in the acid fraction of beef liver lipids.

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## Electrophoretic Patterns of Seminal Plasma from Some "Abnormal" Human Semens.*

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We have previously published[1] some chemical and electrophoretic data on the proteins of normal human seminal plasma. We have since had an opportunity of obtaining semen specimens which were considered "abnormal" in one respect or another. These have been examined electrophoretically and, to a limited extent, chemically as well, to learn whether any differences between the proteins of such specimens and normal ones could be detected. The patterns are recorded here together with several additional ones of normal seminal plasmas.

The earlier work had demonstrated the presence in human seminal plasma of the following electrophoretic components: P1, the proteose fraction ($\mu = -.6 \times 10^{-5}$ cm$^2$ volt$^{-1}$ sec$^{-1}$); P2 and P3, globulins,† each with a water soluble and a water insoluble fraction ($\mu = -2.9$ and $-4.6$ respectively), and probably a third globulin (P2a) with an intermediate mobility. A glycoprotein P4 ($\mu = -5.7$) is also present as well as a still faster moving component P5 ($\mu = -6.3$), which was considered related to P4 and possibly derived from it.‡ P4 and P5 are not always present together in the same specimen.

*Methods and Results.* The methods employed were as previously described.[1] All electrophoretic examinations were done on cell free, centrifuged specimens in phosphate buffer containing 0.055 M NaCl (pH 7.85, ionic strength 0.1) at ca. 2°C.

The electrophoretic patterns 1, 2, and 3 (Fig. 1) are of 3 normal specimens and show the presence of P1 (proteoses), P2 and P3 (globulins), and either P4 or P5 or both (glycoprotein). P2a was present in one of the 3 specimens and in the pooled mixture of 5 normal specimens, as is shown in pattern 4.

The usual peaks P1, P2, P3, and P5 were seen in the patterns, shown in illustrations 5 and 6 in the figure, of 2 abnormally viscous

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1 Ross, V., Moore, D. H., and Miller, E. G., Jr., *J. Biol. Chem.*, 1942, **144**, 667.

† We refer to P2, P2a, and P3 as globulins because there are water soluble and water insoluble fractions of each of these in fresh seminal plasma. If, however, seminal plasma is allowed to stand for several days before dialysis against water, no insoluble fraction separates. The term "globulin" may, therefore, not be accurate.

‡ When acetic acid is added to the water soluble fraction following dialysis of a fresh specimen against water, the precipitate is stringy and is soluble in 0.1% acetic acid containing 1% sodium chloride. If the acetic acid is added to the fresh, undialyzed plasma, the precipitate is not stringy and does not dissolve in this solution. When these operations were carried out on separate portions of the 5 pooled specimens (pattern 4, Fig. 1), the mobilities of the precipitates, following purification by dissolving in sodium hydroxide and reprecipitation with acetic acid, were 6.0 and 7.0 respectively (ascending, 1 hour). The two kinds of acetic acid precipitate, on analysis, yield similar values for nitrogen and reducing substance following hydrolysis.[1] Absorption in the ultraviolet[2] by the two forms is similar also.

2 Ross, V., and Ross, L., in press.