Substances in Bone-Marrow Extract accelerating Coagulation of the Blood

THOUGH previously it has been known that bone marrow contains a hormone-like substance which has an accelerating action upon the blood coagulation[1], its chemical nature has remained unclarified. We have now investigated this problem. Bone marrow from long tubular bones of domestic fowls, after removal of lipids with alcohol, acetone and ether, was extracted with physiological saline and then the extract was deproteinized with sulphosalicylic acid. After removal of excess sulphosalicylic acid with calcium carbonate, the filtrate was concentrated to a small volume. This filtrate showed a remarkable accelerating action on the blood coagulation.

Attempts were then made to separate the active fraction by several methods (absorption, fractionating precipitation with chemicals, etc.) and finally by paper chromatography. The active concentrate was chromatographed on No. 50 Tōyō filter-paper strip (2 cm. × 40 cm.) or on wider paper (40 cm. × 40 cm.) using water-saturated *n*-butanol as the developer (ascending method), and the spots or the bands were observed under ultra-violet light (2537 A.). As shown in the accompanying diagram, three spots, *A*, *B* and *C*, with R_F values of 0·13, 0·26 and 0·38 respectively, and another spot with R_F 0·86 showing a light purple fluorescence, were detected. Moreover, six spots with R_F values of 0·01, 0·05, 0·10, 0·16, 0·29 and 0·33 were detected with ninhydrin, indicating the presence of several amino-acids.

All these bands were cut out separately in nine strips and, after elution with water, each eluate was used in animal experiments for investigating its physiological action. The eluates from *A* and *B* bands distinctly accelerated blood coagulation, while the remaining seven fractions showed no activity.

Fraction *B*, which gave no sugar reaction, proved to be hypoxanthine (see table), because it showed the same R_F value as that of hypoxanthine in water-saturated *n*-butanol and in the two-phase solvent system of 5 per cent potassium dihydrogen phosphate and *iso*amylalcohol (2 : 1), and its ultra-violet absorption spectra in acid and alkaline solutions were identical with that of hypoxanthine. Fraction *A* separated into spot A_1, with R_F value 0·60, and spot A_2, with R_F value 0·69, on developing with the two-phase solvent system of Carter[2]; these R_F values are identical with those of inosine and guanosine respectively. Both fractions A_1 and A_2 gave the orcinol hydrochloric acid reaction[3] and yielded guanine and hypoxanthine respectively on hydrolysing with diluted sulphuric acid.

		Spot *A*		*B*	Guanosine	Inosine	Hypoxanthine	Guanine
		A_1	A_2					
(1) R_F values using *n*-butanol system		0·13	0·13	0·26	0·12	0·14	0·26	
(2) R_F values using two-phase system		0·60	0·69	0·53	0·60	0·69	0·53	
(3) R_F values after hydrolysis (*n*-butanol system)		0·05	0·26	0·26	0·05	0·26	0·26	0·05
(4) Orcinol hydrochloric reaction		+	+	−	+	+	−	
(5) Absorption maxima (mμ)	*p*H							
	1·0	250		248·5	250		249	
	7·0			250			250	
	10·0			261·5			261·5	

In physiological tests using a modified method of Sahli–Fonio with rabbits as test animals, authentic samples of hypoxanthine and guanosine distinctly accelerate the blood coagulation in doses of 200 γ for a rabbit weighing 2·0 ~ 2·5 kgm. The activity of inosine seems a little weaker than that of hypoxanthine or guanosine.

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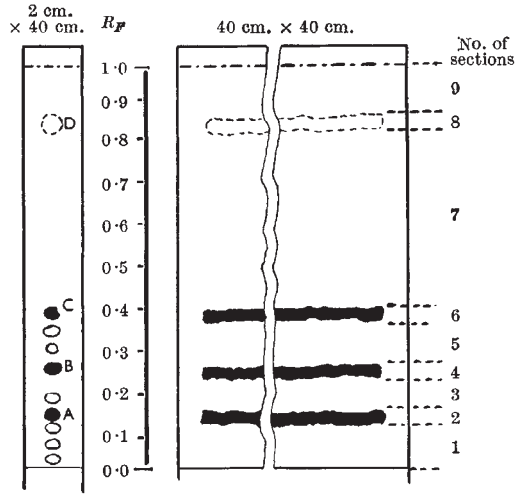

Sub-Diploid Chromosome Variation in Man and other Mammals

THE existence of sub-diploid chromosome numbers in the somatic tissues of mammals has for long been a matter of controversy. This had centred around several earlier reports of the normal occurrence of many somatic cells with less than the diploid number of chromosomes. The controversy has again been recently revived by reports[1] that the human uterine endometrium normally contains cells with varying numbers, ranging from 4 to 104, of chromosomes. The highest frequency peak of these numbers is supposed to lie in cells with 20–25 chromosomes, with a much lower peak in cells with 45–50 chromosomes. It is also suggested that this type of chromosome variation may be a distinct characteristic of warm-blooded animals.

The existence of cells with more than the diploid number of chromosomes would not be a unique occurrence, as different degrees of polyploidy can be present in various tissues. It has also been shown