Nachkriegsjahren als Blutersatz sowie als Vehikelsubstanz[1] Verwendung gefunden.

Kürzlich haben AMMON und Mitarbeiter[2] gezeigt, daß am Hund und am Kaninchen ein Teil des verabreichten Peristons gespeichert wird und in den Organen histologisch nachweisbare Veränderungen verursacht. Nur etwa 40–60% des Peristons werden durch den Harn ausgeschieden.

AMMON und BRAUNSCHMIDT[2] vermuten, daß nur die niedrigmolekularen Anteile des Polyvinylpyrrolidons die Nieren passieren, während die höhermolekularen Anteile vornehmlich in der Milz und in den Lymphknoten abgelagert werden.

Wir haben nun zwei käufliche Injektionslösungen auf ihre Polydispersität hin untersucht, indem wir die Eindampfrückstände nach dem Entsalzen fraktionierten und die Molekulargewichte mit Hilfe von Ultrazentrifugen- und Viskositätsmessungen bestimmten.

Dabei erhielten wir folgende Ergebnisse[3]:

Handelspräparat I		*Handelspräparat II*	
%-Gehalt	Durchschnittsmolekulargewicht	%-Gehalt	Durchschnittsmolekulargewicht
11,8	54000	13,4	76000
13,2	52500	7,8	54000
11,1	35000	11,3	42500
11,1	34000	9,9	28000
15,3	25000	12,7	22500
10,4	19500	8,5	16200
7,0	13200	10,6	12600
4,8	11800	10,6	11000
4,2	8700	2,7	5800
11,1	2700	12,7	5400

Obwohl die Permeabilität der Niere für synthetische Stoffe noch nicht genau bekannt ist[4], scheint uns die von AMMON und Mitarbeitern gegebene Deutung zumindesten für Vinylpolymere in Anbetracht der gefundenen breiten Molekulargewichtsverteilung richtig zu sein.

An der Ratte konnten wir mit Polyvinylalkohol[5] histologisch nachweisen, daß Fraktionen von einem durchschnittlichen Molekulargewicht von 75000 in verschiedenen Organen (Milz, Leber, Niere) reichlicher abgelagert werden als Fraktionen von einem durchschnittlichen Molekulargewicht von 25000.

Da ein Abbau im Organismus unwahrscheinlich und nach den Ergebnissen von AMMON und BRAUNSCHMIDT[6] auszuschließen ist, kann angenommen werden, daß für die Ausscheidung von Vinylpolymeren die Molekülgröße ausschlaggebend ist.

Über Ablagerungsversuche von definierten Polyvinylalkoholfraktionen soll später an anderer Stelle ausführlich berichtet werden.

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Wissenschaftliche Laboratorien der Fa. F. Hoffmann-La Roche & Co. AG., Basel, den 15. Dezember 1950.

Summary

The molecular weight distribution of two commercial blood substitutes of the polyvinyl-pyrrolidone type is determined.

The width of this distribution is discussed in relation to excretion experiments.

It is shown by histological methods that fractions of polyvinylalcohol of an average molecular weight of 75,000 are retained in the organs to a greater extent than fractions of an average molecular weight of 25,000.

New Data on the Physico-chemical Properities of Heparin

One of us (PÁLOS) pointed out that thrombin can be oxidized and becomes in its oxidized form inactive, further that heparin inhibits this oxidation completely in γ-quantities[1].

This hitherto unknown property of heparin induced us to examine the behaviour of heparin (Hoffmann-La Roche) in an oxidizing resp. oxidizing-reducing system. We did not succeed in our past experiments in reactivating the oxidized and thus inactivated thrombin, we made use of the experiments on oxidizing-reducing systems. We chose for our experiments the methylene blue-leukomethylene blue-ascorbic acid-dehydroascorbic acid system and proceeded as follows:

To a solution of methylene blue diluted 1:5000 we added the same quantity of 1% ascorbic acid solution and by boiling reduced the methylene blue to leukomethylene blue. As in our system the quantity of ascorbic acid was in excess of the quantity of methylene blue the system, besides dehydroascorbic acid, also contained ascorbic acid. The ascorbic acid prevented the immediate oxidation of the leukomethylene blue, and therefore our system remained nearly colourless and contained methylene blue only in traces. When heparin in concentration of varying γ-quantities $\frac{(20–200\gamma)}{4–5\,\mathrm{ml}}$ was added to our system the leukomethylene blue underwent oxidation and was reoxidized to methylene blue. The quantity depends on the amount of heparin, whereas the controls without any heparin remained colourless, that is the oxidation of leukomethylene blue did not take place.

We used for measurements PULFRICH's Stufenphotometer.

We had further to clear up whether heparin alters the equilibrium of the leukomethylene blue-dehydroascorbic acid system, whether heparin itself is an oxidizing substance or achieves its effect through transfer of oxygen from other sources. Our experiments proved that heparin does not upset the equilibrium of the leukomethylene blue-dehydroascorbic acid system and is not an oxidizing substance either, but it transfers the oxygen of the air or of any other oxidizing material available, or positive charges, with such vigour that the oxidation of

[1] H. BENNHOLD und R. SCHUBERT, Klin. Wschr. *23*, 30 (1944). – G. BERGOLD, Z. Naturforschg. *3b*, 300 (1948). – R. SCHUBERT, Schweiz. Med. Wschr. *80*, 140 (1950); Deutsche Med. Wschr. *73*, 551 (1948); Ärztl. Forschg. *3*, 425 (1949). – W. KIKUTH et al., Z. Naturforschg. *3b*, 342 (1948).

[2] R. AMMON und G. BRAUNSCHMIDT, Biochem. Z. *319*, 370 (1949). – R. AMMON und W. MÜLLER, Dtsch. Med. Wschr. *74*, 465 (1949).

[3] Eine ausführliche Darstellung der Ergebnisse im Zusammenhang mit der Fraktionierung technischer Produkte erscheint in der Zeitschrift «Die makromolekulare Chemie».

[4] A. GRÖNWALL und B. INGELMANN, Acta Physiol. Scand. *9*, 1 (1945). – E. BAYLISS et al., J. Physiol. *77*, 386 (1933.)

[5] Für den Ablagerungsversuch wählten wir nicht Polyvinylpyrrolidon, sondern Polyvinylalkohol, weil dieser färberisch in den Geweben nachgewiesen werden kann. – W. C. HUEPER, Arch. Path. *34*, 883 (1942); Amer. J. Path. *18*, 895 (1942).

[6] R. AMMON und G. BRAUNSCHMIDT, Biochem. Z. *319*, 370 (1949). – R. AMMON und W. MÜLLER, Dtsch. Med. Wschr. *74*, 465 (1949)

[1] L. A. PÁLOS, Exper. *5*, 207 (1949).

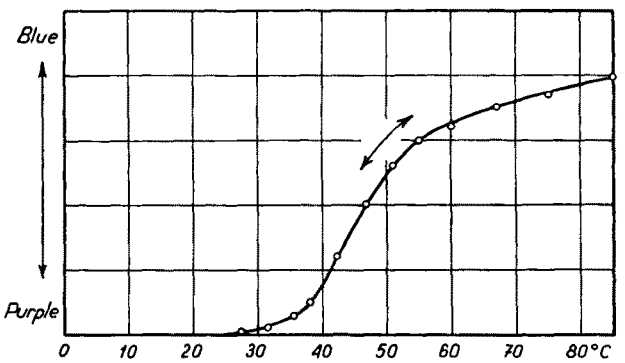
the leukomethylene blue takes place in spite of the presence of ascorbic acid. This fact is proved as follows:

(1) If we make the above-mentioned system containing heparin airtight by a layer of paraffin oil, the effect of heparin on the leukomethylene blue is inhibited completely.

(2) The quantity of leukomethylene blue oxidized by the same amount of heparin depends on the size of surfaces touching the air and the thickness of fluid layers. Mathematically the ratio of the sizes of surfaces referred to the unit of thickness of the layers is equivalent to the ratio of the quantities of oxidized leukomethylene blue. For example: 200 γ heparin oxidized 44 γ leukomethylene blue when the size of surface was 5·5 cm^2 and the thickness of layer 1·5 cm. The same amount of heparin oxidized 7·45 γ leukomethylene blue when the surface was 1·6 cm^2 and the thickness of layer 3·2 cm. The ratio of the quantities of oxidized leukomethylene blue is 5·91. The value calculated theoretically is:

$$\frac{5 \cdot 5/1 \cdot 5}{1 \cdot 6/3 \cdot 2} = 7 \cdot 3.$$

In our experiments the theoretically calculated and experimentally found values agreed as far as 80% (for example: 5·91 and 7·3).



(3) If we added to our system Fe^{+++} ions or H_2O_2 the oxidation of leukomethylene blue was much quicker in the tubes containing γ quantities of heparin than in the control tubes containing no heparin.

The effect of heparin in our system could be inhibited by adding to it small quantities of serum (0·1 ml/5 ml). It has not yet been decided whether this phenomenon is to be explained by the modifying effect of the plasma factor of heparin or by the serum protein causing inactivation of heparin.

We could show the same effect when using toluidine blue instead of methylene blue. This system however, does not yield a pure colour, bluish purple, and was not suitable for colorometric estimation.

The above-mentioned effect of heparin depends much on the p_H. We made our experiments at p_H's between 5–6. The spontaneous oxidation of the leukomethylene blue increases at p_H 7 in control tubes considerably and that of the heparin falls below half. At p_H 7·5, practically no heparin effect can be demonstrated. At higher p_H's there is again a heparin effect, but it could not be measured by colorometric methods, as the colour became greenish blue. In strong basic solutions no spontaneous oxidation could be observed.

As to the mechanism of the heparin effect, it seems probable that heparin with a high molecular weight and high negative charge adsorbes on its surface the basic molecule of leukomethylene blue so that the latter becomes very sensitive for oxidative influences. This supposition is supported by further experiments.

The colour-reaction of heparin-toluidine blue that LISON considered as a specific reaction of sulphuric esters of high molecular weight[1] disappears at higher temperatures and reappears at lower temperatures. As the figure shows, the decomposition begins already at 30° C, and the original purple colour takes a bluish shade. The change of colour between 38–40° C becomes very definite, but complete only at 85° C when the solution is entirely blue. While cooling, the blue turns through transitory colours purple again. Different alcohols (methyl-, æthyl-, propyl-, and butylalcohol, glycerol) and the watery solution of sodium choleinicum also brake the toluidine blue-heparin attachment. If we add propyl alcohol to the system the decomposition begins already at 5% concentration and becomes complete in the presence of 30% of propyl alcohol.

As adsorptive forces decrease and even cease on higher temperatures, and as the desorptive property of alcohols and sodium choleinicum solutions are generally known, our supposition that the heparin toluidine blue attachment is an adsorption seems to be proved.

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Zusammenfassung

Wir untersuchten die Heparinwirkung auf Redoxsysteme und stellten dabei fest, daß selbst sehr kleine Mengen Heparin die Oxydation des Leukomethylenblaues fördern. Das Heparin als ein großes, negativ geladenes Molekül gibt diese Wirkung durch die Adsorption und die dadurch hervorgerufene Aktivierung des Leukomethylenblau-Moleküls. Für den Ablauf der Oxydation ist eine Sauerstoffquelle notwendig. Außerdem wurde bewiesen, daß die Heparin-Toluidinblau-Bindung eine adsorptive Bindung ist.

[1] L. LISON, C. r. Soc. Biol. *118*, 821 (1935); Arch. Biol. *46*, 599 (1935); Bull. Soc. Chem. Biol. *18*, 225 (1936).

Lyophilization and Mutation in Yeast

Four years back, chromosomal and genic changes were observed in yeast cultured in a cold room[1]. This was to be expected since temperature shocks have been known to have different effects on animals and plants[2]. Gene mutations in animals are accelerated by temperature shocks while the same agency induces a doubling of the chromosome complement in plants[3]. In yeasts, gene mutations[4], chromosal translocations[5], and tetraploidy[6] have been observed as a result of thermal shocks. The question arose whether lyophilization may not produce similar changes[7]. The most important step during the process of lyophilization is to cool the culture suddenly[8]

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[2] H. J. MULLER, Gen. *13*, 279 (1928). – H. H. PLOUGH, Biol. Symp. *6*, 9 (1942). – TH. DOBZHANSKY, *Genetics and the Origin of Species* (Columbia Univ. Press, 1941).

[3] H. DERMEN, Bot. Rev. *6*, 599 (1940).

[4] M. K. SUBRAMANIAM, B. RANGANATHAN, and S. N. KRISHNA MURTHY, Cellule *52*, 39 (1948).

[5] M. PREMA BAI, Curr. Sci. *16*, 316 (1947).

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[8] L. J. WICKERHAM and A. A. ANDREASEN, Wallerstein Lab. Commun. *5*, 165 (1942).