isolated which had the same solubility properties. A substance of similar activity also has been isolated from a crude lipid extract of spinal cord of cattle, which was kindly placed at our disposal by Dr. D. Klein, The Wilson Laboratories, Chicago, Illinois.

The organs were dehydrated with acetone and freed of sterols, fats, lecithin and cephalin by exhaustive extraction with acetone and ether. It is essential to remove the ether-soluble phosphatides as completely as possible, since otherwise the inhibitor may be overshadowed by the cephalin which, as is well known, activates blood clotting. The organ powder is then repeatedly extracted at boiling temperature with ethyl alcohol or a mixture of three parts of methyl alcohol and one part of chloroform. The crude material is dissolved in a mixture of two parts of chloroform and one part of ethyl alcohol. A first crop of cerebrosides is obtained on cooling of the solution and further batches are collected by stepwise concentration of the mother liquors.

When tested according to the technique recently described,[2] the first two or three cerebroside crops thus isolated usually show an inhibiting effect on the clotting of chicken plasma, and all but the weakest preparations also markedly inhibit the clotting of blood and of chicken plasma activated by addition of muscle extract. On the basis of its solubility properties a concentration of the active fraction is possible. It is insoluble in acetone, little soluble in cold pyridine and ether, easily soluble in cold glacial acetic acid and chloroform, and can be recrystallized from methyl alcohol or ethyl acetate. These properties indicate that the inhibitor accompanies the sphingomyelin fraction, whereas cerebron and kerasin are devoid of activity. That sphingomyelin itself does not exert the inhibiting effect can, however, be shown by the fact that sphingomyelin preparations purified by precipitation with Reinecke salt[3] are inactive. Our purest preparations contain N and P, but only small amounts of S. It appears highly improbable that heparin, which has entirely different solubility properties, is the active constituent of this lipid inhibitor. In Tables I and II examples of the action of preparations from sheep brain and beef spinal cord are given. The inhibitor from sheep brain is one of the strongest obtained so far.

It may be relevant to point out that the question as to whether heparin is the physiological agent which controls the fluidity of blood is by no means settled.

TABLE I

LIPID INHIBITOR FROM SHEEP BRAIN

Mg in 0.1 cc of plasma or blood	Clotting time minutes		
	Activated chicken plasma	Recalcified oxalated human plasma	Human blood
0	9	2	4
0.031	36	..	...
0.062	54	8	...
0.124	90	18	100
0.249	> 250	51	150
0.498	> 250	82	...

TABLE II

LIPID INHIBITOR FROM BEEF SPINAL CORD

Mg in 0.1 cc of plasma	Clotting time minutes	
	Chicken plasma	Activated chicken plasma
0	97	7
0.10	135	9
0.20	225	14
0.39	255	18
0.78	345	25

In order to isolate heparin from tissue comparatively drastic means are necessary. We have found in experiments which have not yet been published that, when a mild method of extraction is employed, it is impossible to liberate heparin even from liver, in which it is known to occur in considerable amount. It may be that the lipid inhibitor described above will prove of interest in connection with the problem of clotting inhibitors contained in blood and in thrombocytes.[4]

The work here described is being continued and will be published in detail at a later date.

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THE DECOMPOSITION OF YEAST NUCLEIC ACID BY A HEAT RESISTANT ENZYME

IN the course of a study of the action of different extracts of animal tissues upon pneumococci, preparations have been obtained which exhibit a high degree of enzymatic activity upon yeast nucleic acid.[1] The enzyme has been prepared from polymorphonuclear leucocytes and from several organs, especially the liver, pancreas, spleen and lungs of different animal species. It possesses certain interesting properties, which are the same irrespective of the source from which it is prepared.

The enzyme, a polynucleotidase, is remarkably resistant to heat, with a zone of maximum stability

[2] E. Chargaff, F. W. Bancroft and M. Stanley-Brown, *Jour. Biol. Chem.*, 115: 149, 1936.

[3] S. J. Thannhauser and P. Setz, *Jour. Biol. Chem.*, 116: 527, 1936.

[4] E. Chargaff, F. W. Bancroft and M. Stanley-Brown, *Jour. Biol. Chem.*, 116: 237, 1936.

[1] The author is indebted to Dr. P. A. Levene for supplying him with a sample of yeast nucleic acid.

around pH 4 to pH 5; even at pH 1.5 and pH 7.5 the enzyme can be heated in a boiling water bath for five minutes with only little loss of activity. The rate of action upon yeast nucleic acid increases with temperature up to 75° C.; it then decreases sharply and no appreciable action can be detected at 85° C. When an enzyme-substrate mixture is maintained at 95° C. no enzymatic hydrolysis occurs; if, however, the same mixture is now brought back to a temperature compatible with enzymatic activity (60° C., for instance), the nucleic acid is rapidly decomposed, indicating that the inhibiting effect of high temperatures upon the enzyme-substrate mixtures is completely reversible.

The enzyme appears to be a protein; it is readily salted out in saturated sodium sulfate solutions; it is rapidly decomposed by pepsin, but is very resistant to trypsin and chymotrypsin.

After being acted upon by the polynucleotidase, the yeast nucleic acid becomes soluble in mineral acids and glacial acetic acid; the purified preparations of the enzyme, however, do not release any inorganic phosphorus from yeast nucleic acid or indeed from any phosphoric esters tested. In other words, the enzyme does not behave as a phosphatase. No action could be detected upon thymus nucleic acid.

In 1913 Jones[2] stated that he had observed in a preparation of digested pancreas the existence of a principle capable of breaking down yeast nucleic acid into dinucleotides. This observation does not seem to have been confirmed and the same author himself stated later that "it has been found difficult to repeat this experiment."[3] It appears, however, that the enzyme described in the present paper may be the same as that discovered by Jones.

As stated elsewhere, the same enzyme preparations which decompose yeast nucleic acid are also capable of rendering heat-killed pneumococci Gram negative.[4] It appears possible that the same agent is responsible for both types of actions, since the effect of temperature, of trypsin and of pepsin is common to both reactions. Furthermore, it has been possible to extract from pneumococcus cells a soluble fraction which reacts like nucleic acid and which is readily decomposed by the enzyme.

Finally, it may be stated that several samples of crystalline trypsin and chymotrypsin[5] have been found to contain small amounts of a heat-resistant principle which attacks both yeast nucleic acid and heat-killed pneumococci. Both types of action could be completely eliminated by repeated recrystallizations of the proteolytic enzymes.

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[3] W. Jones, *Monographs on Biochemistry*, 37, 1914.

[4] R. Dubos and C. M. MacLeod, *Proc. Soc. Exp. Biol. and Med.* In press.

[5] The author is indebted to Drs. J. H. Northrop and M. Kunitz for supplying him with several samples of crystalline trypsin and chymotrypsin.

THE PREPARATION OF CRYSTALLINE β-4-GLUCOSIDOSORBITOL AND ITS NONOMETHYL DERIVATIVE

RECENTLY Karrer and Büchi[1] reported on the reduction of cellobiose to β-4-glucosidosorbitol. They failed to obtain the substance in crystalline form or analytically pure. In view of the importance which this method of catalytic reduction is acquiring in the domain of sugar chemistry, we wish to report on the preparation of β-4-glucosidosorbitol in form of beautifully crystalline platelets, melting at 133° C. and having a specific rotation in water, $[\alpha]_G^{25} = -8.7°$.

The reduction of the cellobiose was carried out in aqueous solution under pressure of 100 atmospheres in the presence of Raney's catalyst, the temperature being 75° during 8 hours of each day, the remaining part of each day the temperature remaining at about 25°. The operation lasted 48 hours.

The composition of the substance was C 41.83, H 7.21. Calc. C 41.83, H 7.03.

A single methylation with dimethylsulfate under conditions of West and Holden gave an exhaustively methylated product, which distilled at 170° and 0.2 mm pressure. The specific rotation of the substance was $[\alpha]_D^{25} = -4.93°$ (absolute ethanol) and the composition of the substance was C 53.34, H 9.01, OCH_3 58.91. The theory required C 53.37, H 9.00, OCH_3 59.32.

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