

in press), on the isolation of crystalline vitamin B₁₂, which appears to be the pure anti-pernicious anaemia factor.

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A New Extraction Procedure for the Preparation of Heparin

THE usual method of preparing heparin, due to Charles and Scott¹, involves rough extraction of tissues with alkali and subsequent tryptic digestion of the protein precipitate.

Special investigations, which will be reported elsewhere, made it highly desirable to prepare heparin by means of milder extraction methods avoiding the use of alkali. For this purpose, a number of salt solutions of different concentrations were tested. A 10 per cent calcium chloride solution, as used by Meyer *et al.*² for the extraction of chondroitin sulphuric acid, proved to be ineffective, and so did several other salt solutions. It was found that solutions of potassium thiocyanate exert a pronounced power of extracting fairly quickly and completely the mast cell granular substance from various tissues (liver and skin). Using *M* potassium thiocyanate solution for twenty-four hours, we obtained about 90 per cent extraction of the metachromatic material in ox liver. Histochemical examination of the tissue residues (cf. Sylvén³) showed only insignificant amounts of metachromatic substances left after such extraction. Metachromatic material was not found to adhere to other constituents, such as collagen, as it often does when previous methods are used.

After the extraction, the potassium thiocyanate was readily removed by dialysis. The following fractions then remained to be analysed: tissue residue, precipitate, supernatant and dialysate. The different fractions were treated by tryptic digestion according to Charles and Scott, and the heparin contents were assayed by estimating the anti-coagulating effect by means of the thrombin method according to Jaques and Charles⁴. The following amounts of heparin expressed in mgm. 'standard heparin' according to Jorpes⁵ were found:

Amount of heparin obtained from ox liver

Tissue residue	5.5 mgm./kgm.
Precipitate	56.3 "
Supernatant	0 "
Dialysate	0 "
Total	61.8 "

Obviously, the extracted substances displaying heparin activity were precipitated following the removal of thiocyanate. The heparin precipitated during dialysis seems to be bound to proteins. The complex is split by trypsin treatment, and afterwards almost all the heparin can be recovered. The collected yield tallies well with that reported by Wilander⁶ (56-75 mgm./kgm. ox liver).

The properties of heparin prepared in this way will be described elsewhere.

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Effect of Thyroid Deficiency on the Level of Pseudo-Cholinesterase in the Plasma of Rats

THE effect of thyroid deficiency on the level of pseudo-cholinesterase in rat plasma has been examined in the following experiments.

Adult male Wistar rats (200-400 gm.) were used. They were maintained on a diet of chow ('Purina') which has been shown to be adequate for normal growth in the rat colony. All animals were killed by exsanguination through the jugular vein, and plasma from the oxalated blood was used for estimating pseudo-cholinesterase activity. The determinations were carried out by the method previously described¹. Group I consisted of twenty-seven normal animals which served as controls. Group II was made up of sixteen animals which survived thyroidectomy. In this operation the removal of varying amounts of parathyroid tissue could not be avoided. To offset the effects of parathyroidectomy, daily subcutaneous injections of 2 ml. of a 5 per cent calcium gluconate solution were given for a period of seven days. The animals were killed fourteen days after operation; this time interval seems sufficient for the recovery of the level of pseudo-cholinesterase from the transient decline resulting from surgical intervention². To exclude the possibility that the effects observed were due to parathyroidectomy rather than thyroidectomy, a third group of fifteen rats was treated with thiouracil. These rats received for a period of 23-27 days a water ration containing 0.06 per cent thiouracil. With an average daily intake of 20 ml. of water, the thiouracil consumed by each rat was thus in the neighbourhood of 12 mgm. a day.

It will be seen from the figures in the accompanying table that thyroidectomy and treatment with thiouracil cause an increase in the level of the pseudo-cholinesterase of the plasma.

LEVEL OF ACTIVITY* OF PSEUDO-CHOLINESTERASE IN THE PLASMA OF MALE RATS

Group	Normal Thyroidectomized Thiouracil-treated		
	I	II	III
Number of animals	27	16	15
Average activity	41.0	97.1	117.3
Standard error	2.3	8.7	10.6

* μ l. Carbon dioxide evolved by 1 ml. plasma in 20 minutes. Substrate: benzoylcholine chloride (0.006 *M*).

A statistical analysis of the results obtained reveals that the probabilities for the differences in pseudo-cholinesterase activity arising on a basis of pure chance