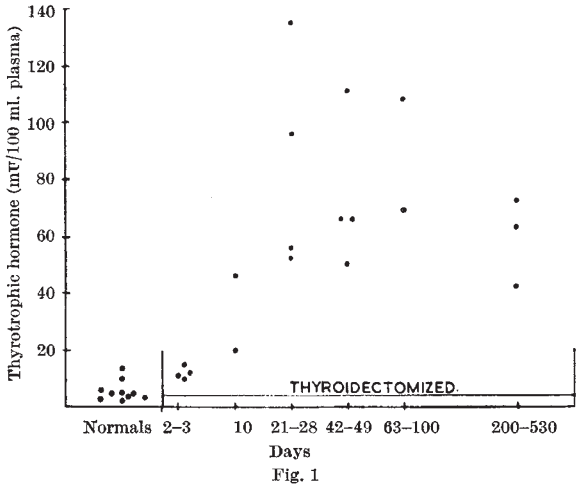

Fig. 1

International milliunits of thyrotrophic hormone per 100 ml.

The mean λ was found to be $0{\cdot}115 \pm 0{\cdot}002$ in a series of consecutive assays. The F ratio for the slope was highly significant in all valid assays, P varying between $< 0{\cdot}05$ and $< 0{\cdot}001$ (Table 1). There was no significant deviation from parallelism between International Standard thyrotrophin on one hand, and the thyrotrophic hormone content of human plasma, that of normal rat plasma, and that obtained from the thyroidectomized rabbit. In a series of 30 consecutive assays undertaken on specimens of blood obtained from normal and thyroidectomized rabbits, all tested blindly, only two assays proved to be statistically invalid, and hence had to be repeated. The results were quite consistent with what might be expected on a theoretical basis, namely, that the thyrotrophic hormone content of the blood of the rabbit rises gradually after thyroidectomy (Fig. 1).

It is suggested that this new method (Bottari, Donovan and El Kabir, to be published) is suitable for the assay of thyrotrophic hormone in blood.

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[1] Bottari, P. M., and Donovan, B. T., *J. Physiol.*, **140**, 36 P (1958).
[2] Bliss, C. I., *The Statistics of Bioassay* (Academic Press, New York, 1952).

PHARMACOLOGY

Anti-Heparin Agents as Inhibitors of Plasma Kinin Formation

Plasma kinin formation[1] can be induced by treating plasma in a variety of ways, excluding the addition of proteolytic enzymes. In our present work the procedures which we have applied to 'pre-active' human plasma or serum include exposure to a large glass surface[2], addition of ε-amino-caproic acid or disodium edetate[3], dilution[4], re-suspension of the 33 per cent saturated ammonium sulphate precipitate, or addition of the purified kinin-forming material isolated from human plasma (Armstrong, D., and Mills, G. L., unpublished work).

Having observed that heparin[1] and dextran sulphate (Armstrong, D., and Stewart, J. W., unpublished work) accelerate plasma kinin formation, we decided to determine the actions of heparin antagonist drugs on kinin production. We found that both protamine sulphate, and also the non-protein polymer hexadimethrine bromide[5–7] ('Polybrene', Abbott Laboratories, Illinois):

$$\left\{\left[-(CH_2)_6-\overset{CH_3}{\underset{CH_3}{N^+}}-(CH_2)_3-\overset{CH_3}{\underset{CH_3}{N^+}}-\right]\cdot 2Br^-\right\}_x$$

mol. wt. average 6,000 effectively antagonized kinin formation induced by all the aforementioned methods. Toluidine blue, on the other hand, was relatively inactive. Protamine sulphate and hexadimethrine bromide antagonized not only the accelerating action of heparin on kinin formation but also acted as potent inhibitors in the absence of heparin, that is, when production was being induced in citrated plasma, or in serum. They did not interfere with the uterine response to formed plasma kinin, and did not increase the activity of kininase in plasma. Since we have found no evidence of action on substrate, kininogen (in pre-active plasma or serum), their action is apparently on the kinin-forming enzyme itself.

Effective concentrations of protamine sulphate and hexadimethrine bromide are in the range 10^{-5}–10^{-8} gm./ml., the latter being about 50 times the more active of the two compounds. Antagonism of plasma kinin formation induced by glass is an exception, in that higher concentrations of antagonist are required, for example, 10^{-4}–10^{-3} gm./ml., due to the fact that concentration of antagonist in solution is reduced by adsorption on to the glass.

We suggest that the actions of these positively charged macromolecular inhibitors, protamine sulphate and hexadimethrine bromide, resemble those of the naturally occurring inhibitor of kinin formation present in normal human plasma and that the actions of the negatively charged heparin and dextran sulphate molecules resemble those of glass in sequestering off this natural inhibitor from the kinin-forming system.

These agents should assist *in vivo* studies to determine the physiological role of the plasma kinin-forming system.

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[1] Armstrong, D., and Stewart, J. W., *J. Physiol.*, **154**, 19, P (1960).
[2] Armstrong, D., Jepson, J. B., Keele, C. A., and Stewart, J. W., *J. Physiol.*, **135**, 350 (1957).
[3] Armstrong, D., Keele, C. A., and Stewart, J. W., *J. Physiol.*, **150**, 20, P (1960).
[4] Schachter, M., *Brit. J. Pharmacol.*, **11**, 111 (1956).
[5] Egerton, W. S., and Robinson, C. L. N., *Lancet*, ii, 635 (1961).
[6] Preston, F. W., Hohf, R., and Trippel, O., *Quart. Bull. Northwestern Univ. Med. School*, **30**, 138 (1956).
[7] Weiss, W. A., *J. Amer. Med. Assoc.*, **166**, 603 (1958).

Changes in the Blood of the Rat induced by the Monocarboxylic Acid of Cyanocobalamin obtained by Fermentation (anti-Vitamin B_{12})

Monocarboxylic acid derivatives of vitamin B_{12} obtained by mild acid hydrolysis are competitive B_{12} antagonists in *Escherichia coli*. The three isomeric