Addendum

MARTHE VOGT

DR ROBERTS has asked me whether I would add a warning note about some of the pitfalls which should be avoided in catecholamine estimations which make use of chromatography in phenol-HCl followed by bioassay. Though these warnings have all been published, they are somewhat scattered, so that a recapitulation might be helpful.

HEAT TEST

Plasma extracts. "The phosphates and traces of other chemicals which are present in the eluates may affect the isolated uterus and mimic or mask the presence of traces of adrenaline. Thus the volume of eluate added to the uterus bath has to be kept below the threshold of such interfering substances. The threshold is established by preparing an eluate from a strip of paper not containing any adrenaline. It was usually well above the quantity of eluate required for an assay. In cases of doubt, the neutralized solution was heated for 5 min. in a boiling water bath, so that any adrenaline was destroyed, and the responses to the heated solution were examined without and with added adrenaline" (Vogt, 1952).

Tissue extracts. In extracts of brain tissue heating was extended to 10 min at pH 8 in a stoppered tube sealed with adhesive tape (Vogt, 1954).

VOLUMES OF ELUATE

To minimise effects of phosphate and hypertonic solutions, volumes of eluate should not exceed 2 or 2·5 ml 0·4% NaH_2PO_4. Elution time will control the volume of eluate. The residue is taken up in 0·5 ml distilled water which gives an approximately isotonic solution. Only if more than one tenth of the solution is needed for assay is there serious danger of effects from interfering substances.

CONTAMINATION WITH PHENOL

An eluate of, say, 2·5 ml 0·4% NaH_2PO_4, diluted to 3·5 ml with distilled water (used for rinsing the collecting tube), can be completely freed of phenol by evaporation to dryness *in vacuo*. If neither the tube containing the residue not the stopper smell of phenol the residue is perfectly safe; the stopper, however, may have taken up some phenol and may require washing before it can be used to seal the tube.

OVERLOADING OF PAPERS

An error which is obvious to any chemist but not always to the biologist is that of overloading the papers to be chromatographed. There may be overloading with the catecholamines themselves, as when adrenal medullary extracts are prepared, or with other tissue constitutents, such as the lipids

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referred to by Dr. Roberts. Thus it is not feasible to apply the extract of much more than 5 ml plasma to a paper lane 12 cm long. If lipaemia is present, the normal procedure may have to be modified.

CONTAMINATION

When very sensitive bioassays are used, particularly in the tests for isoprenaline, it is essential to cut off the strips of paper carrying control spots before washing the remainder of the paper in benzene. Traces of the control substances may otherwise contaminate the benzene and spread over the whole of the paper (Muscholl & Vogt, 1958).

Vogt, M. (1952). *Brit. J. Pharmacol.*, **7**, 325–330.
Vogt, M. (1954). *J. Physiol.*, **123**, 451–481.
Muscholl, E. & Vogt, M. (1958). *J. Physiol.*, **141**, 132–155.