response to adrenaline stress is not abolished. It seems, therefore, that although the 'block' produces an increase of circulating eosinophils, the functional state of the reticulo-endothelial system has no influence on the eosinopænic response observed in stress.

Detailed hæmatological and bone-marrow studies of rats treated with trypan blue will be published elsewhere.

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Extraction of Acetylcholine from Brain Tissue

IN some recent attempts[1] to follow rapid changes in the concentration of acetylcholine in mammalian brain *in vivo*, the experimental animals were killed by freezing in liquid air, a method which had already yielded valuable results when applied to the study of certain other labile metabolites[2]. As an accompaniment to these studies, one of us[3] reported that the amount of acetylcholine which can be extracted from excised brain frozen in liquid air is some 13 per cent greater than when it is extracted from unfrozen tissue by the more usual methods. Since this work was completed, however, Elliott and his colleagues[4,5] have claimed that a complete extraction can be achieved only by homogenizing the unfrozen brain in an acid saline solution; according to these workers, previous freezing of the excised tissue results in a loss of up to 50 per cent of its contained acetylcholine. It has, however, proved impossible to confirm these findings, as the following experimental results testify.

Acetylcholine was extracted from the excised brains of adult rats, using the method described by Elliott *et al.*[4], except that the extracts were assayed in the frog rectus muscle. While histological examination showed that the homogenization had been efficiently carried out, the amount of acetylcholine extracted corresponded to a brain concentration of only $1{\cdot}69 \pm$ S.E. $0{\cdot}12$ μgm. per gm. (ten animals). A second series of brains was extracted by a similar method, using a bladed homogenizer (10,000 r.p.m.), and was found to contain $1{\cdot}73 \pm$ S.E. $0{\cdot}03$ μgm. acetylcholine per gm. (four animals). These figures are very much lower than those reported by Elliott and his co-workers, namely, $3{\cdot}45$ μgm. per gm., and are similar to those usually quoted for excised tissue. It seems possible that the leech muscle used by the Canadian workers was affected by sensitizing substances. MacIntosh[6] found that saline extracts of mouse brain do exert a sensitizing effect on the leech preparation, though Elliott and his colleagues[4] deny that their extracts had a similar effect. Tobias and his co-workers[7] used an extraction technique similar to that of Elliott *et al.* but assayed their preparations on the frog rectus muscle, which is known to be very susceptible to the effects of sensitizing substances in brain extracts. Though they failed to eliminate the effects of these agents and must therefore have obtained an exaggerated estimate of the amount of acetylcholine present in their extracts, their results are very similar to those quoted by Elliott *et al.* In the present experiments, it was found that, for saline extracts of brain, an acetylcholine assay of $1{\cdot}8$ μgm. per gm. rose to $2{\cdot}9$ μgm. per gm. (mean of six experiments) when the effect of the sensitizing substances was not controlled, though the rectus muscles were very sensitive and had been treated with optimal amounts of neostigmine.

In other experiments, excised brain tissue was divided into two equal portions, one of which was homogenized in acidified saline solution, while the other, after freezing in liquid air, was powdered and its acetylcholine extracted by contact with aqueous trichloroacetic acid for twenty minutes. The homogenized brain was found to contain $1{\cdot}38 \pm 0{\cdot}03$ μgm. acetylcholine per gm., the frozen brain $1{\cdot}40 \pm 0{\cdot}11$ μgm. per gm. (four experiments).

There is thus no evidence that freezing is associated with a loss of acetylcholine; it is possible that the results of Elliott and Henderson[5] were due to their allowing some thawing of their frozen tissue before its acetylcholinesterase had been completely inactivated, for acetylcholine disappears very rapidly from frozen tissue if even momentary thawing is allowed to occur[3].

Finally, the acetylcholine content of the brains of a series of sixteen adult rats, killed by immersion in liquid air, was found to be $2{\cdot}52 \pm 0{\cdot}15$ μgm./gm., while the excised brains of litter mates of five of these animals contained only $1{\cdot}82 \pm 0{\cdot}02$ μgm./gm. There seems to be no loss of acetylcholine from excised brain during the first few minutes after decapitation, and it would appear that the low content of acetylcholine in the excised brain resulted from the effects of the violent stimulation accompanying the process of decapitation. It is evident that such an effect could mask any but marked differences in the concentration of acetylcholine in the brains of animals subjected to different experimental procedures. For the detection of such differences, as well as for the determination of the absolute acetylcholine content of the brain, the use of liquid air would seem to remain the method of choice.

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Passive Transfer of Transplantation Immunity

PASSIVE transfer of transplantation immunity has never been successfully achieved in a simple way. Previous demonstrations of passive transfer have either employed special techniques giving results which are difficult to interpret[1,2], or else the nature of the immunity has been in doubt[3]. Here is reported