and the ^{14}C-activity in aliquots of wash fluid determined by standard scintillation counting technique. The loss of ^{14}C-label from the tissue was by then at a steady and low level. Trypsin (Koch-Light) was added for 2 min at a concentration of $2{\cdot}5 \times 10^3$ BAEE units/ml and washed out. A further six washes were collected and the output of ^{14}C-label determined in each. As may be seen in Fig. 1 the treatment with trypsin caused an increased output of specific activity. This episode coincided in time with the partial restoration of the response of the muscle to noradrenaline. The washes immediately before and after trypsin were concentrated at 50° by passing a warm air draught over the surface and run for 8 h on Whatman 3 MM paper in butanol: glacial acetic acid: water 50:12:25 as solvent. The front was marked and the dried paper exposed to Ilford Industrial G X-ray film in the dark for three months. The spot on the paper corresponding to radio activity on the film was ninhydrin positive, Rf 24·2. This result is interpreted to mean that suitable treatment with trypsin removes from guinea-pig vas deferens previously treated with SY28 a small peptide to which the 2-haloalkylamine is bound and that this restores its lost sensitivity to noradrenaline, possibly by uncovering an undamaged noradrenaline - receptor which was previously occluded by the blocking drug. Species differences may well require variation in the detail of enzyme treatment.

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The effects of stress and intravenous 0·9% NaCl injection on concentrations of whole brain 5-hydroxytryptamine in the neonate chick

Alterations in amounts of brain biogenic amines can occur under certain conditions of stress. Barchas & Freedman (1963) reported elevation of 5-hydroxytryptamine (5-HT) and lowering of noradrenaline in the brain of rats subjected to cold and swimming stress. Similarly, Goldberg & Salama (1969) observed an increase in rat brain 5-HT after stress with either electric shock or a revolving drum, although cold exposure alone did not produce this alteration.

The newly hatched chick and the newborn guinea-pig are functionally competent in the first hours of life. These two species do show a significant difference in that the guinea-pig has a well developed blood-brain barrier at birth, whereas the neonate chick does not and is considered to be neurologically immature (Waelsch, 1955; Lajtha, 1957; Key & Marley, 1962). Both however, are capable of surviving some forms of stress.

The basis of our present report occurred, initially, with the observation that one day old chicks entered a continuous roosting state when subjected to the stress of plucking neck feathers, making a small incision in the skin, or injecting 0·9% NaCl into the jugular vein. This was in contrast to the behaviour of untreated birds which

Table 1. *Effect of stress on levels of whole brain* 5-HT *in the neonate chick*

Treatment	5-HT (μg/g)	% change	N
None	1·28	—	25
Stress + 0·9% NaCl	1·53*	+19·72	21

* $P < 0.05$ analysis of variance.

displayed a cyclic pattern that consisted of alternation between states of roosting and arousal. The behaviour of the stressed animals was quite similar, but less pronounced, to that obtained in chicks injected intravenously with 5-HT (Seifter, Rauzzino & Kramer, 1963; Kramer, Kobrin & Seifter, 1963).

To determine whether an alteration in levels of brain 5-HT was occurring, this amine was estimated fluorimetrically in both stressed and non-stressed chicks using the procedure of Bogdanski, Pletscher & others (1956). The results (Table 1) indicate that stress, accompanied by roosting behaviour, does induce a significant increase in whole brain 5-HT of approximately 20% over control animals. This increase is almost identical to that found in rat brain by Barchas & Freedman (1963).

Thierry, Fekete & Glowinski (1968) have shown that stress of electric shock in rats not only causes a small but significant increase in endogenous 5-HT, but also greatly increases its turnover with the rate of amine synthesis being somewhat greater than catabolism. This partially supports the findings of Gál, Heater & Millard (1968) who showed significant increases in cerebral, but not liver, tryptophan-5-hydroxylase during cold exposure without significant increase in endogenous 5-HT. Bliss, Ailion & Zwanziger (1968) demonstrated stress-induced changes in indole metabolism as manifested by a significant increase of 5-hydroxyindoleacetic acid in all major areas of the rat brain, without appreciable change in levels of endogenous 5-HT. It would seem from the work cited that the major effect of stress on the central serotonergic system is stimulation of turnover of 5-HT with sedation as the behavioural correlate. It is questionable, at this point, whether endogenous levels of 5-HT in rat brain are actually altered during this process.

The newly hatched chick with its permeable blood brain barrier does however have the potential for increasing brain levels of 5-HT when this amine becomes elevated in peripheral blood.

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