calculated as percent difference or actual difference. In the case of pentagastrin the fall off in responses for protease and protein was significantly less following duodenectomy than after an equal time lapse without operation.

Atropine (Figure). Atropine, 3 mg/animal, significantly depressed the response of amylase, protein and portease to vagal stimulation by approximately 80%. It did not alter the responses to CCK or pentagastrin.

Discussion. HICKSON[2] has shown that vagal stimulation is effective in enterectomized, anesthetized pigs but has not shown whether or not it is less so than in intact animals. He has shown, as we have, that atropine does not reduce juice volume but does reduce enzyme secretion.

In these studies we have controlled the decline in gland responsiveness with the passage of time, and also, by using i.v. CCK and pentagastrin before and after duodenectomy, the effect of the surgery. It is evident that surgery has at least as profound an effect on the responses to CCK and pentagastrin as on vagal stimulation. The reduced response to vagal stimulation after duodenectomy is more than offset by the spontaneous loss in gland sensitivity with the passage of time and by the decline in sensitivity to CCK and pentagastrin following duodenectomy. We have been unable, therefore, to produce evidence in the chloralose-anesthetized pig that vagal stimulation releases either secretin or CCK.

Zusammenfassung. Beim Schwein wird mittels Parameter des Pankreas nachgewiesen, dass Sekretin- oder auch Cholecystokinin-Sekretion vom Vagus reguliert wird.

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[10] Acknowledgement: We are grateful for the help of R. Pfahler, G. Rice and R. Williams.

A Quantitative Investigation of the Response to Injury of the Central Nervous System of Rats Treated with ACTH and Triiodothyronine

Although interruption of a tract in the mammalian central nervous system (CNS) is not usually followed by any functional regeneration, histological signs of axonal growth and/or indications of some return of function have been claimed following the administration of the thyroid hormones triiodothyronine (T3) and tetraiodothyronine (T4),[1,2] and adrenal corticoids[3,4] or substances which cause their release, such as ACTH[1,5] or the bacterial polysaccharide 'Pyromen'[3,6–9].

Most workers consider that circulating corticoids stimulate the phagocytic activity of macrophages, depress the cellular and fluid phases of inflammation and decrease the formation of connective tissue at the site of a wound in the CNS and thereby facilitate regeneration by providing an environment through which axons grow more easily. On the other hand thyroid hormones may promote regeneration by increasing protein synthesis in central neurons[1].

This paper reports the findings of a quantitative study on the effects of ACTH and T3 on the glial response within the corpus callosum following surgical incision.

Materials and methods. Adult male Wistar rats aged 40 days post partum were used. 30 animals were allocated to each of the following 4 treatment groups: 1. normal saline; 2. ACTH (Synthecin Depot CIBA); 3. T3 (Glaxo Freeze Dried preparation); 4. ACTH and T3 together. Within each group 5 animals were allocated for study at 1, 2, 5, 10, 50 and 100 days after cutting the corpus callosum. Injections were given 6 h before making the lesion and at 24 h intervals thereafter. The total number of injections received by each animal surviving for a period of 1, 2, 5, 10, 50 or 100 days were 2, 3, 6, 7,7 or 7 respectively. The doses given (per 100 g body weight) were as follows: 0.75 ml normal saline; 10 μg ACTH; 3 μg T3; 10 μg ACTH + 3 μg T3 (see FERTIG et al[1] for rationale of dosages). The entire corpus callosum was cut stereotaxically along a saggittal plane 2 mm from the midline.

The glial reaction occurring in the corpus callosum 1, 2, 5 and 10 days after making the lesion was measured counting the number of cells in 5 sections from each animal, occupying a grid 75 μm × 75 μm placed 50, 150, 250, 500 and 1000 μm from the boundary of the wound in sections stained with cresyl violet. As well as estimating total density, the cell population was differentiated into seven sub-populations, namely: light oligodendroglia, medium oligodendroglia, dark oligodendroglia, astrocytes, microglia and endothelial cells according to their nuclear characteristics[10–14] and 'cytoplasmic cells' according to both the configuration of chromatin and the presence of a stainable cytoplasm (a group of cells probably analogous to 'brain macrophages').

The functional tests for regeneration of axons consisted of eliciting an interhemispheric response (IHR) in 2 groups of 5 animals surviving for 50 and 100 days after making the lesion. The IHR is mostly eliminated by cutting this tract except for a characteristic low amplitude residual response[15]. Qualitative histological examination of silver stained sections from the brains of these animals were also carried out.

The activity of the ACTH and T3 were tested by measuring the release of corticosterone using a fluori-

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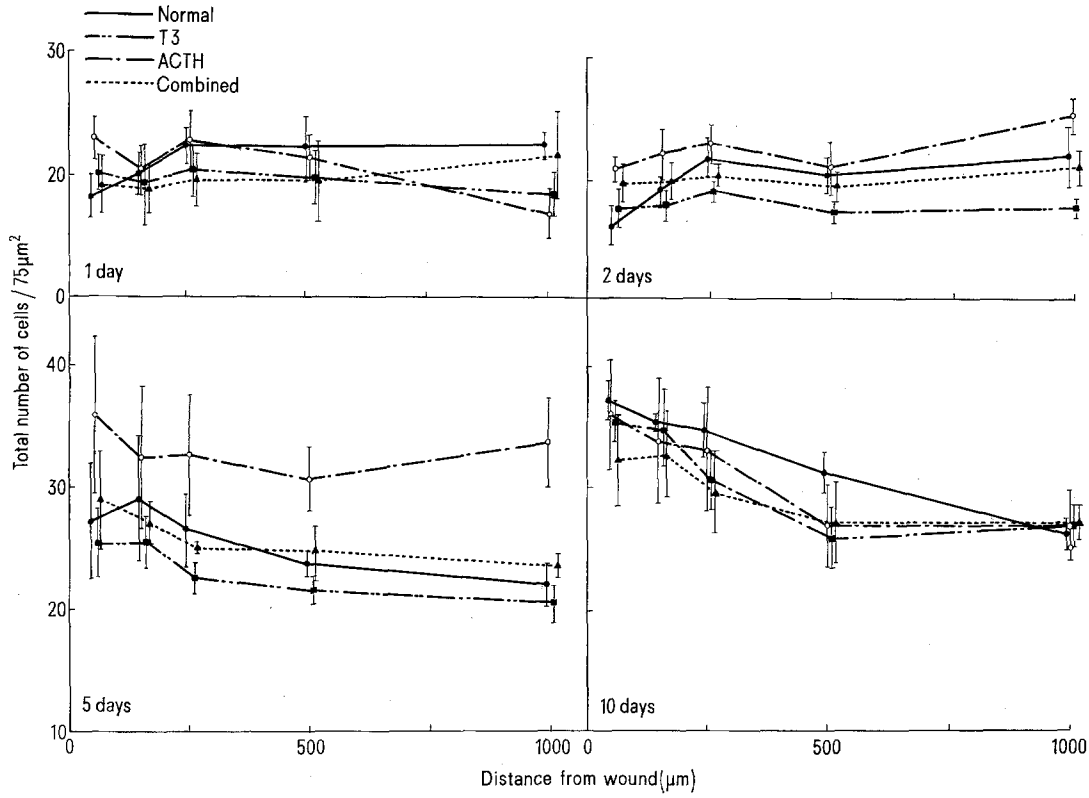

Fig. 1. The reaction of the total cell population in the corpus callosum 50, 150, 250, 500 and 1000 μm from the boundary of the lesion, 1, 2, 5 and 10 days after operation in rats injected with normal saline (Normal) T3, ACTH and T3 + ACTH (Combined).

metric technique[16] and the uptake of I^{131} sodium iodide in the thyroid glands respectively. Control animals received injections of normal saline.

Results. It was found that none of the treatments with hormones caused any change in the time course or magnitude of the reaction of any of the cell types when compared with the reactions in control animals. Figure 1, shows the reactions of the cell population as a whole and demonstrates this finding clearly. Most individual cell types in the corpus callosum reacted to injury in a characteristically similar manner; initially their numbers dropped near the site of the lesion to values below those recorded at a distance but soon recovered and by 10 days the number near the lesion was consistently greater than the number at a distance. The one exception to this pattern of response was the reaction of 'cytoplasmic cells'. On the first post-operative day 'cytoplasmic cells' were seen in larger numbers near the edge of the wound than at a distance and this pattern persisted throughout the 10 day period of study.

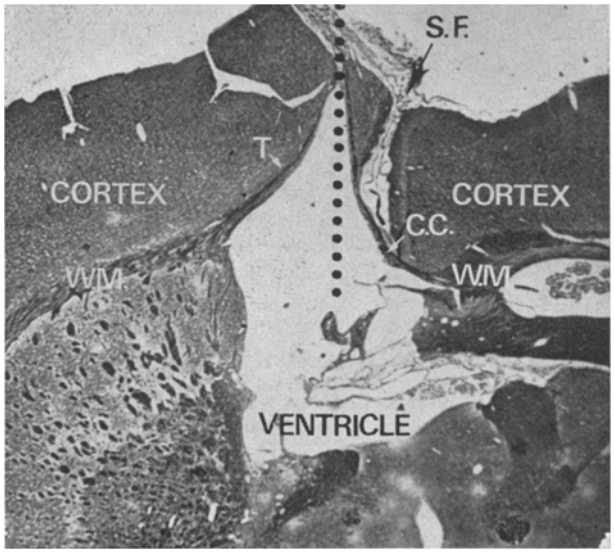

Fig. 2. Photomicrograph of a coronal section (7 μm thick) of the brain of a rat showing the site of the lesion on the 50th post-operative day. Note the cavitation of the wound and the tract (T) of fibres which lines the cavity (Glees-Marsland × 16). S.F., Sagittal fissure; W.M., White matter; C. C., Corpus callossum; the dotted line marks the line of the incision.

The electrophysiological tests for regeneration carried out at 50 and 100 days failed to elicit any response which was clearly indicative of the growth of axons across the lesion. Histological examination of specimens revealed that regeneration had not occurred because of the presence of a large cavity between the cut ends of the tract. In a number of cases this cavity was lined by a tract of axons (Figure 2). It was considered that the tract was created by shrinkage of the damaged cortex rather than regrowth of axons along the edge of the wound. This proposition is explained in Figure 3. The ACTH and T3 assay procedures showed that in both cases the hormones were physiologically active.

Discussion. In the light of these results, it seems unlikely that ACTH and T3 induce regeneration in the CNS by way of altering the glial reaction or the number of 'cytoplasmic cells' that collect at the site of injury. The

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validity of these conclusions is supported by the fact that the preparations of hormones used in these experiments were shown to be physiologically active at the dosages used. This finding substantiates the work of CAVANAGH and JOSEPH[17] and challenges the accepted ideas about the mode of action of ACTH in promoting regrowth of axons[13,14]; strongly suggesting that an alternative explanation for the ability of these substances to induce regeneration must be sought.

FERTIG et al[1] showed that both T3 and ACTH can promote regeneration in the mammalian CNS. However it was not possible to demonstrate regeneration in the present experiments simply because the leakage of CSF from the ventricle into the incision produced a cavity which could not be bridged by axons.

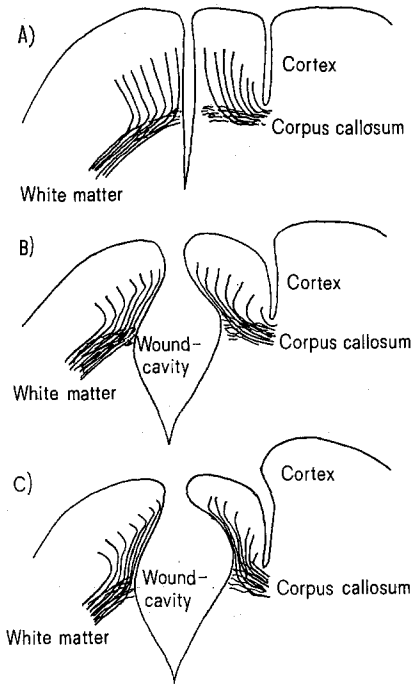

Fig. 3. Proposed explanation through A to C. for the formation of the tract of axons seen to lie along the edges of the wound in animals sacrificed 50 and 100 days after operation.

Résumé. Une étude quantitative des effets de ACTH et T3 sur la réaction des cellules gliales dans le corpus callosum, après incision, a montré que ces 2 hormones n'ont aucun effet sur cette réaction. Ainsi, on ne peut plus soutenir l'idée généralement acceptée que ces hormones provoquent une régénération partielle de l'axone central du système nerveux, en modifiant la cicatrice gliale.

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Iproniazid Interaction with the H^3Norepinephrine Uptake and Retention, on Isolated Left Atrium of Guinea-Pig

In a early paper from our laboratory, iproniazid (IPN) was found to be a blocking agent of the H^3norepinephrine (H^3NE) uptake by isolated ventricle of frog[1]. However, it was desirable to elucidate if this blockade was produced at the neuronal membrane ($uptake_1$) or intraneuronally, namely, by diminishing the re-entry of H^3NE into the storage vesicles present in sympathetic nerve endings.

In other studies on isolated atria of guinea-pig (unpublished results), we observed that the increase of H^3NE uptake between reserpinized atria, treated with IPN, with respect to their controls without the MAO inhibitor, was very much higher than the increase of the H^3NE uptake observed in no-reserpinized atria, treated with IPN and their untreated controls. These observations are in agreement with those reported by other investigators[2]. The facts prompted us to think that IPN blocked the re-entry of H^3NE into storage vesicles, so that this blockade was only present in unreserpinized atria, diminishing the difference in the incorporation of H^3NE between the atria treated with IPN and their untreated controls.

If we accept this hypothesis the treatment with IPN could produce a disturbance in the turnover of NE at the sympathetic nerve endings, by diminishing the inflow and by maintaining unchanged the outflow. Consequently, the normal rate of NE from the storage vesicles will decrease. In the present paper, it has been studied whether IPN blocks the incorporation of H^3NE into storage vesicles present in the nerve endings of the isolated atrium of the guinea-pig.

Methods. The experiments were carried out with guinea-pigs of either sex weighing from 500 to 800 g. The left atrium was isolated and mounted as previously described by FURCHGOTT et al.[3]. In each experiment one half of the atrium served as a control and the other half as the experimental preparation. The Krebs-bicarbonate solution used contained 10^{-5} g/ml of ethylene diamine tetraacetic acid (EDTA) and 10 m*M* of glucose through which 95% O_2 and 5% CO_2 or 95% N_2 and 5% CO_2 was continuously bubbled. Each half was subjected to a resting tension of 1 g and was electrically driven at a rate of 30 beats/min. Atria were attached to force displacement transducer (Grass, model FT03) and mechanical activity was recorded by a Grass polygraph.

Under their respective conditions, halves were then incubated with 5 ng/ml of d, l-H^3NE (specific activity, 16, 7Ci/mmol, New England Nuclear Corp.) for 5 min and then thoroughly washed. 4 additional washes were given over the subsequent 40 min period at the end of which

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