the differentiation of cambial derivatives into roots. It may not be out of place to mention here that even starch can be used as a source of carbon for the supply of energy required for root initiation, and that it is mobilized into sugar by the enzymes that leach out of the segments into the medium[7].

Another interesting point that emerges from these results is that the auxin enhances the effectiveness of exogenously applied nucleic acids. The results thus lend support to the postulate that auxin probably acts as a triggering agent at the transcription level, and nutrition serves as a source of carbon to regulate translation[8]. The results are of particular significance in the light of evidence which suggests that the exogenously supplied DNA and RNA effectively enter in the intact cells and protoplasts of various eukaryots[9]. The regulated uptake of exogenous DNA molecules in the cells of plant origin and their expression is also suggested by the work of some others[10–14]. Infact, LESHEM and GALSTON[15] showed that RNA which is extracted from tobacco pith cells and is vaccuum-infiltrated into similar receptor cells, alters the pattern of isoperoxidases in the receptor tissue.

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Spread of Cobalt from a Cortical Epileptic Lesion Induced by a Cobalt-Gelatine Implant into the Frontal Cortex of the Rat

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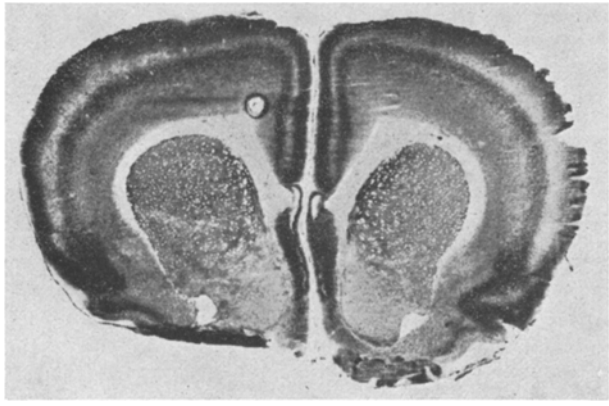
Summary. The spread of cobalt ions from cobalt induced epileptic foci in rats has been investigated. Atomic absorption spectrophotometry and heavy-metal histochemistry reveal cobalt ions spread very widely from the focus. Biochemical and physiological consequences for this model of epilepsy are discussed.

KOPELOFF et al.[2] first reported that the application of powdered cobalt metal to the frontal cortex of the monkey produced epileptiform spikes in the electroencephalogram (EEG). Since KOPELOFF's original observation the application of cobalt powder to the cortex or the insertion of cobalt gelatine pellets into brain has been used to produce reproducible epileptic foci in a variety of animals[3]. In the rat, application of cobalt to the cortex produces a distinct secondary focus in the contralateral cortex[4,5]. Because of the use of cobalt salts to trace axonal pathways[6] we were interested to know if the secondary focus, formed in the contralateral cortex of the rat did contain significant amounts of cobalt. If this were so then the value of this model would be reduced. Previous workers using this model[4,5] have suggested that the secondary focus in this model arises as a response to the spread of electrical signals from the primary focus across the corpus callosum and represents a response similar to the kindling phenomenon described by GODDARD[7]. The presence of significant amounts of cobalt in the secondary focus would mean that it is probably solely caused by the presence of cobalt ions.

Cobalt-gelatine pellets prepared as described by FISCHER et al.[8] of standard size 1 mm diameter and maximally 0.5 mm thick (representing at most 1 mg of cobalt metal in gelatine) were inserted into the right frontal cortex of male PVG rats as described in detail by Dow et al.[4]. The histochemical distribution of cobalt ions after this implant was investigated using the TIMM[9] staining method at 4, 28 and 60 days after implantation. The rats were anaesthetized with an overdose of barbi-

A



A) The distribution of sulphide silver positive material (heavy metals) in the frontal cortex as revealed by the TIMMS method. Note the uneven distribution of staining in the cortical layers.

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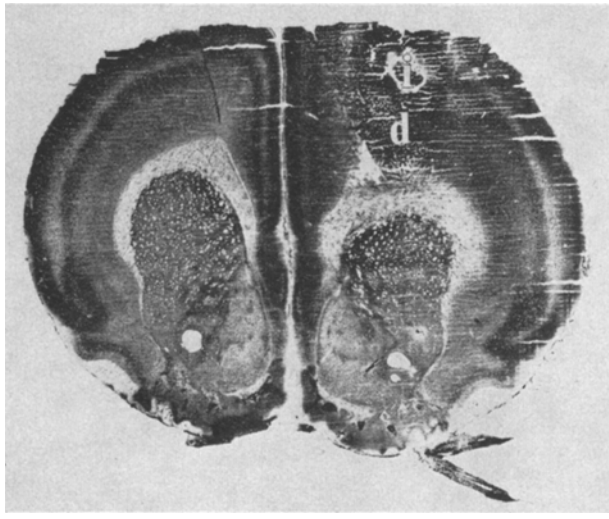
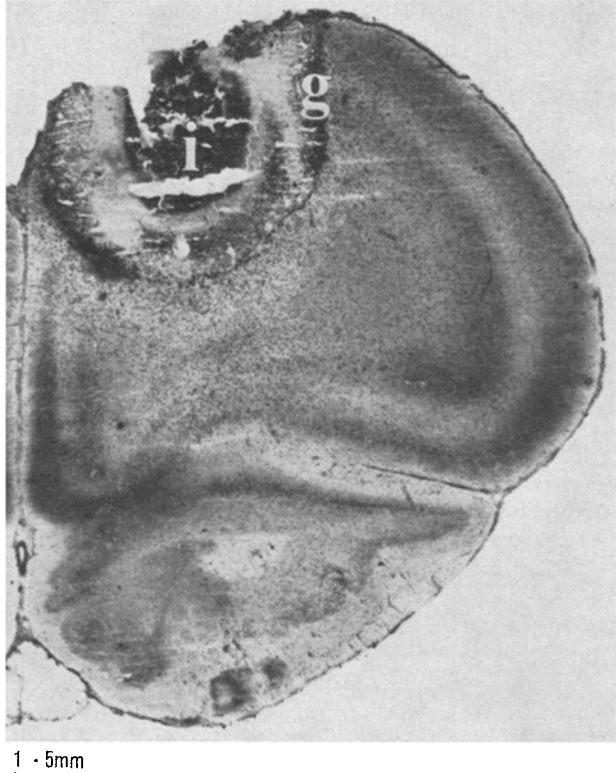
B



B) The effect of a cobalt-gelatine implant on the TIMM-staining pattern of the frontal cortex. The rat was sacrificed 4 days after the operation to implant the cobalt. Note the marked increase in staining around the lesion, extending even in to the underlying caudate nucleus. In the secondary focus (contralateral cortex) the staining pattern seems more intense than in control sections incubated for the same time (Figure A). However it was not possible to be certain that there was definite increase in staining in the contralateral cortex.

C



C) A section through the centre of an established cobalt-epileptic focus (28 days survival). Note the definite calcified glial capsule surrounding the lesion, seeming to act as a barrier to further diffusion of cobalt. There is no longer any evidence of increased staining around the lesion and in contrast there is a decrease in TIMM positive material outside the glial capsule.

Key to symbols on Figures B and C. d, area of cobalt diffusion; i, cobalt implant; g, glial capsule.

turate and perfused transcardially with phosphate buffered sulphide solution. Cryostat sections of the frozen brains were serially mounted and impregnated by physical development as described by HAUG[10]. The results, of applying the TIMM-staining method to the frontal cortex of rats 4 days after implantation revealed a marked increase in the density of histochemical staining around the cobalt-gelatine implant (Figure B) up to 2–3 mm in each direction from the original implant. Increased staining was evident in the cingulate cortex, corpus callosum and caudate nucleus below the lesion. We could demonstrate no unequivocal increase in staining in the contralateral cortex or secondary focus and the staining pattern in the contralateral cortex does not differ significantly from control sections (Figure B). The laminar pattern of stainable material agrees with previous observations[10] but the significance of the staining pattern is not understood. By 28 or 60 days (Figure C) the primary focus has become surrounded by a calcified glial capsule and no histochemical evidence of cobalt spread outside the glial capsule is evident. In fact by 28 days the glial capsule seems to provide a barrier to further spread of cobalt and this may account for the regression of the epileptic focus[2,11]. At no time during the development and regression of the cobalt focus could we be certain from histochemical staining that there was a spread of cobalt ions into the contralateral cortex, although we suspected this was occurring.

In order to further investigate the possible spread of cobalt we used atomic absorption spectrophotometry. This method would allow unequivocal demonstration of cobalt ions in the contralateral cortex if these were present. A Perkin-Elmer atomic absorptiometer, model HGA 360 was used and samples were read at a wavelength of 240 nm. Weighed pieces of brain were digested in concentrated HNO_3 and 20 µl of digest representing 1 mg of tissue was injected into the furnace. Using this program no loss of cobalt was detected during either the evaporation or ashing stages. No traces of cobalt were found in the reagents or incubation vessels. Known amounts of cobalt 1–50 ppm were taken through the procedure to provide a standard curve against which tissue data could be evaluated.

The results of these determinations are shown in the Table. Notice particularly the atomic absorption results show clearly that cobalt spreads extremely widely from the lesion site. At day 6 cobalt is found in significant amounts in the secondary focal area, in the occipital cortex both ipsi- and contralateral to the lesion site and also in the raphe nuclei some 1.5 cm from the cortical lesion site. The results are consistent with cobalt ions diffusing or being transported away from the original lesion to establish a gradient of cobalt through the brain. The amount of cobalt which spreads by diffusion is not likely to be very extensive outside the original lesion and it is probable that the majority of cobalt is spread to the rest of the brain by axonal transport. In agreement with this suggestion the levels of cobalt in the contralateral caudate follow closely the levels in the overlying frontal cortex with which it has connections. By day 21 the cobalt levels in the contralateral frontal cortex are significantly lower (1.8 ± 1.4 ppm) than the cobalt levels in the contralateral occipital cortex (5.3 ± 4.7 ppm) probably as a result of the extensive degeneration of neurones, axons and terminals in the ipsilateral frontal cortex and consequent reduction in transport. At day 21 there is

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Cobalt concentrations in ppm (dry matter) ± SD of the mean

	Cortex				Caudate nuclei		Raphe nuclei
	Frontal		Occipital				
	Lesion	Contralateral	Ipsi-	Contralateral	Ipsi-	Contralateral	
Day 6	>50 ppm	10.5±5.5	16.8±7.5	12.1±6.2	19.9±5.3	10.0±6.6	3.0±1.9
Day 21	>50 ppm	1.8±1.4	7.9±4.7	5.3±4.7	6.1±3.9	1.98±0.8	2.4±1.6
Day 97	No detectable cobalt						

There were 5 rats in each group. In all cases the glial capsule and any visible traces of cobalt-gelatine were removed from primary lesion samples. It was often not possible to remove all traces of the cobalt-gelatine, however, and therefore physiologically inactive cobalt may well have been assayed in the primary lesion. Excess values referred to represent values of over 50 ppm and were not quantified. Control rats and 97 day rats were assayed under the same conditions as the day 6 and 21 rats (see text) and under these condition no cobalt could be detected.

still significant cobalt in the brain outside the implant. However by day 97 there is no longer detectable cobalt in brain areas outside the glial capsule.

The implication of these results, for this and related models of epilepsy, are profound. Although the in vitro levels of cobalt (2–10 μM) which would be obtained in preparing a conventional brain homogenate (e.g. 1 mg brain tissue in 10 µl buffer) from an animal with a cobalt implant would not be sufficient to inhibit in vitro the enzymes we have previously studied (e.g. tyrosine hydroxylase choline acetyltransferase, glutamic acid decarboxylase)[12]. However, the levels in vivo (20–100 μM) will undoubtedly interfere with a wide range of metabolic process. In fact the wide spread of the cobalt ions and the possibilities of their concentration in different cellular compartments, such as nerve terminals suggests that a large area of the brain may become epileptogenic rather than just a small area around the original implant. These results indicate the need for caution in using heavy metal implants as models of epilepsy and suggest that the basis for cobalt induced epilepsy probably lies in a certain selectivity of cells, terminals and enzymes to the toxic effects of this ion in vivo. Certainly the secondary focus produced in this model of epilepsy although very interesting biochemically cannot be regarded as being untouched by the toxic effects of cobalt.

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Increased Aggression in Rats after Withdrawal of Long Term Used Oxazepam

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Summary. The withdrawal of oxazepam (5 mg/kg i.p.) applied for 1 year in rats, increased shock-induced aggression of animals. This phenomenon is interpreted as a sign of abstinence and suggests that long-term treatment causes dependence to oxazepam in rats.

Oxazepam has a central pharmacological profile consisting primarily of anticonvulsant, sedative-hypnotic, muscle relaxant and anxiolytic activities[2–4]. It is an effective ataractic benzodiazepine drug for treating anxiety in patients[5]. The data in the literature concerning its antiaggressive effects are controversial. In this report we present evidence that oxazepam given for 1 year to rats had no antiaggressive action, but withdrawal of long-term drugs intensifies instrumentally induced aggression.

Methods. Experiments were carried out on male rats of Wistar strain from the Central Animal Farm of Silesian School of Medicine. The weight of animals at the beginning of the experiment was 135–145 g; 5 rats were housed in a cage of dimensions 33 × 40 × 26 cm. During the whole experiment the animals had free access to standard laboratory diet and water. Rats were treated for 1 year with oxazepam (Polfa) at a dose of 5 mg/kg i.p. every day except Sundays. Control animals were injected i.p. with 0.9% NaCl solution, used as a solvent. Drug or solvent were applied in the volume of 1 ml/kg. Aggressive behaviour of rats was induced by applying electrical foot-shock according to Eichelman[6]. The pair of rats was placed in Plexiglas box of dimensions 32 × 25.5 × 30.5 cm with stainless-steel grid floor. Shock was delivered by a constant current at 2 mA for a duration of 0.4 sec every 7.5 sec. For the evaluation of shock-induced-aggression, rats were subjected to daily sessions of testing consisting of 50 foot shocks to each pair of rats. The pairs of the same treated or control rats were examined always be-

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