activities of this oxygen function. The suggestive correlation between certain pharmacological results and purely chemical considerations is at the moment tenuous, since many more examples are needed before such inductions can command our confidence. These correlations should, however, encourage us to perform experiments designed to confirm or invalidate the tentative theory outlined above.

Speculation is often sterile, and in the biochemical field inductive arguments from incomplete data are particularly prone to be upset by even small additions to that data. When however a suggestive arrangement of existing data can be made, and no other is yet obvious, it does no harm to examine the consequences of such an arrangement. The application of William of Occam's Razor suggests that the hypothesis that oxidation/reduction at C-11 may be the basis of the biological action of the cortico-steroids is not unworthy of experimental trial, but no one, least of all the author, will be surprised if once again William's shave is postponed by the blunting action of a Baconian Experiment.

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Zusammenfassung

Es wird versucht, die möglichen Zusammenhänge zwischen den physiko-chemischen Eigenschaften der C_{11}-Gruppe, dem Stoffwechsel und der biologischen Wirkung der 11-Oxysteroide aufzuzeigen. Die Bedeutung der reversiblen Oxydationreduktion dieser Gruppe wird besonders hervorgehoben.

On the Intraneural State of Acetylcholine*

By O. Loewi[1], New York

From experiments following soon after the discovery of the chemical transmission of nervous impulse it was concluded that the primary action of the impulse propagated in cholinergic nerves consists in increased synthesis by the nerve-endings of ACH (Acetylcholine) which subsequently is released[2]. Later it was demonstrated that the nerve-endings during nerve stimulation do indeed synthetize ACH but only secondary to its release and in an amount just compensating for the loss[3]. In fact, intact cholinergic nerves even under conditions such as isolation and rest, where continuous destruction and resynthesis is most improbable, maintain a fairly constant level of ACH in spite of the simultaneous presence of cholinesterase. The apparent protection from esterase is regarded as an indication that the ACH is present in the organs in an indiffusible state preventing contact with the esterase. The extent of this protection and the difference in fate between freely diffusible ACH and that present in an isolated, inactive organ is clearly shown in experiments[4] in which the ventricle of a frog heart was divided in two. The ACH was determined immediately in one half and after an hour in the second half: the values were found to be identical. In another experiment ACH was added at the start to the second half. After an hour the ACH content was found to be equal the original value, indicating that only the added diffusible ACH was destroyed.

There arises the question of whether the total content of ACH is indiffusible also in non-isolated, resting organs. The answer to this question is necessary for recognizing the activities by which the nervous impulse leads to the release of ACH: if the whole ACH is present within the nerve and especially its endings in an indiffusible state, the nervous impulse has to render part of it diffusible and to release it. If a part is diffusible, the nervous impulse has only to release it from the nerve-endings. There exist so far no conclusive experiments on the state of ACH–by the way also of epinephrine–in the respective nerves, because of the inadequacy of the methods used. It therefore cannot yet be decided which of the alternatives just mentioned is realized.

In order to fill this gap it seems advisable to investigate whether the state of ACH within nerves could be disclosed by application to nerve homogenates of the method of differential centrifugation. This method, by which mitochondria could be isolated for the first time, has lately been used to separate from organ homogenates granules that fix in an inactive, indiffusible state highly active, endogenous amines such as the

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pressor amines in the adrenal medulla[5] and histamine in liver, spleen and lungs[6]. The granules appear, like mitochondria, to be surrounded by a semipermeable membrane, since it has been shown that influences, interfering with the integrity of living membranes, such as hypotonicity of the medium, freezing and thawing, or surface active agents (e.g. saponin and lysolecithin) cause the release of catechol amines or histamine from the respective granules. Moreover, the sedimentation properties of these granules are very similar to those of the mitochondria. It has therefore been suggested that at least a large portion of the particles may be identical with the latter.

Quantitative determination of the particulate portion of the amines in the adrenal medulla, lung and liver revealed that it amounted to 70 to 90% of the whole amine content of the homogenates. It is possible that part of the amines may be free also in the living cells–perhaps in equilibrium with the fixed portions. On the other hand it cannot be excluded that the whole content of amines in living cells may be fixed, the small part found free in the homogenates being due to artefacts such as mechanical disruption of granules during their preparation or to the inappropriate character of the suspending fluid. The latter was found to be true for example for isotonic Ringer solution, an adequate medium for intact cells. This is not astonishing since the ionic composition of this solution differs widely from that of the cytoplasmic fluid that normally surrounds intracellular structures. It might be worthwhile to check a suspending fluid whose ionic composition would correspond to that of the cytoplasm. Such a medium would seem preferable to the quite generally used sucrose solution, which, in spite of its unphysiological character, has so far proved to be superior to any other suspending fluid for the isolation and preservation of mitochondria and the aforementioned other particles.

I have called attention to the results obtained with catechol amines and histamine in order to justify the previous recommendation that the method of differential centrifugation should be applied to the problem of the intraneural state of the amine ACH.

We now have to refer to the outcome of experiments, devoted to this question in the past. In these the organs were simply extracted with eserinized, neutral Ringer solution. After extraction of the finely minced central nervous system of frogs[7] or the homogenized brain of rats[8] the bulk of ACH was found in the insoluble residue. And by extraction of the brain with hypotonic solution the greater part of the fixed ACH was released[9]. This effect of hypotonicity of the medium suggested that the ACH in the brain is largely if not entirely located in membrane-surrounded particles perhaps identical with mitochondria[10].

Quite different results were obtained with a peripheral cholinergic nerve. When the vagus taken from freshly slaughtered cattle was immediately minced and extracted in the same way as the central nervous system, no ACH was found in the insoluble residue[10]. This finding, however, is not definite proof against the occurrence in the intact nerve of particulate matter containing ACH, since particles from peripheral nerves might not withstand exposure to Ringer solution as well as particles from brain. It would certainly be desirable to apply to nerve homogenates the method of differential centrifugation which has disclosed the presence of particles in cases where other methods had failed.

Whereas brain and peripheral nerve differ greatly in the extractability of their ACH by Ringer solution they behave identically on extraction with acidified aqueous fluids. They extract their total ACH. The same holds true for extraction with acidified alcohol (m/100 HCl-alcohol). This, however, becomes evident only after treatment of the dried alcoholic extract with acidified aqueous solution[7]; but after suspension of the alcoholic extract in Ringer solution, one detects in the filtrate only about 40% of the total ACH content of the organs[11]. One therefore has to conclude that the major portion of ACH is present in the alcoholic extract in a water insoluble, inactive complex. This complex is soluble in ether, in which ACH *per se* is almost insoluble; after the evaporation of the ether the ACH can be extracted from the residue by aqueous solutions[12].

A behaviour analogous to that of ACH was later shown for the catechol amines and histamine: VON EULER[13] demonstrated that catechol amines, present in an alcoholic extract of adrenergic nerves or added to an ethereal solution of lecithin or cephalin, became partly soluble in ether and could be extracted from it by aqueous solutions.

Quite recently LINDAHL[14] found in *in vitro* experiments that also the ether insoluble histamine combines

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with the aforementioned phosphatides to form ether soluble complexes.

Because of the similarity between the above findings and the behaviour of ACH it is probable that also the latter forms complexes with phosphatides.

The observations we just referred to obviously do not justify the conclusion that amine-phospholipid complexes are formed also in the living cells. It could be that the lipids and the amines are simultaneously extracted from tissues as separate entities and that the complexes are formed only when they accidentally meet *in vitro*. On the other hand, the possibility exists and has been discussed[15] that water-insoluble amine-phosphatide complexes are formed also *in vivo*. In favor of this view one could refer to the fact that phosphatides are widespread in the tissues and that the catechol amines fixing granules in the adrenal medulla were found to be especially rich in phospholipids; in fact they amounted in the granules to 50% of the phospholipid content of the whole medulla[16].

It was mentioned before that one factor responsible for the indiffusible state of the amines, contained in particles, might be that these presumably are surrounded by a semipermeable membrane. Another contributing factor, perhaps related to this could be the presence at or in the granules of amine-phospholipid complexes which might be easily split under conditions leading to release of amines[17]. Here changes of the pH may play an important part, because it has been shown that the distribution of catecholamines[18] and histamine [14] between the solid, aqueous, and ethereal phases depends largely upon the prevailing pH.

This essay started from the question of the intraneural state of ACH. The answer had to be restricted to the presentation of possibilities. I have stressed the individual data from which they emerged, in the hope that some of the reflections may indicate directions for future progress in the field.

Zusammenfassung

Durch differenzierende Zentrifugierung von homogenisierten Organen wurde neuerdings nachgewiesen, dass die stark wirksamen, körpereigenen Amine Histamin, Adrenalin und Noradrenalin in Partikeln enthalten sind, die offenbar von halbdurchgängigen Membranen umgeben sind. Reagenzglasbefunde machen es ferner wahrscheinlich, dass diese Amine mit Phosphorlipoiden wasserunlösliche Komplexe bilden. Es sind bereits Anhaltspunkte dafür vorhanden, dass das in nervösen Organen vorhandene Acetylcholin sich den anderen Aminen analog verhält. Sollte sich durch künftige Versuche, für deren Durchführung gewisse Vorschläge gemacht werden, diese Annahme als richtig erweisen, so wäre die Unfähigkeit des Acetylcholins, in ruhenden Organen zu diffundieren, und damit seine Inaktivität und sein Schutz gegen die Esterasewirkung erklärt.

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Présence d'uraninite dans les minéraux accessoires du granite de baveno

Dans le cadre d'une étude sur la distribution de la radioactivité dans les roches éruptives par la méthode photographique[1], nous avons trouvé dans le granite de Baveno (Alpes italiennes), des inclusions microscopiques dont la radioactivité α correspond à une teneur en Uranium supérieure à 50%. La présence de minéraux accessoires aussi actifs dans un granite a été rarement signalée[2] et présente un grand intérêt dans le problème de la métallogénie de l'Uranium. Sur une surface totale de 400 cm^2 (lames minces et sections polies) exposée à des émulsions Ilford C 2, on a trouvé 6 inclusions de surfaces comprises entre 10^{-6} et $8{,}4 \cdot 10^{-5}$ cm^2 dont l'activité spécifique est en moyenne de 200 α/cm$^2 \cdot$ s (mesurée sur les cristaux les plus grands pour être dans les conditions d'émission en couche épaisse).

Cette activité est due à la famille de l'Uranium. En effet, sur 30 000 α émis par les 6 inclusions, nous n'avons trouvé aucune trajectoire dont le parcours dans l'émulsion est supérieur à 39 μ (l'α du RaC' a un parcours de 36 μ, celui du ThC' est de 47 μ). Le rapport Thorium-Uranium est donc inférieur à $5 \cdot 10^{-3}$. L'absence de Thorium est confirmé par le rayon des halos pléochroïques entourant deux de ces inclusions dans la biotite. La concentration en Uranium de ces minéraux calculée par les

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