the possibility that the increased acetylcholine-levels could be due to an increased rate of production of acetylcholine due to chemical or physical stimulation.

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Substances controlling the Rate of Beating of the Heart of *Periplaneta*

A paper now in the press[1] shows that the heart accelerating factor from the corpus cardiacum of the cockroach *Periplaneta americana* described by Cameron[2,3] acts by stimulating the pericardial cells to produce a second substance. It is this pericardial cell factor which is responsible for the direct stimulation of the heart. This communication describes the properties of the two substances.

The factor from the corpus cardiacum appears to be a peptide or protein. Heating extracts (prepared by grinding corpora cardiaca in insect Ringer or in distilled water) on a boiling water-bath for 5 min. results in a slight increase in activity as measured on the isolated cockroach heart. More prolonged heating has no further effect on the activity. The activity of extracts is unaffected by evaporation to dryness at room temperature and resuspension in distilled water. The dried residue can be extracted with *n*-butanol, acetone or diethyl ether without loss of activity. The active factor appears to be soluble in 80 per cent ethanol, but treatment of the dried residue with absolute ethanol destroys the activity. Incubation of extracts with trypsin at *p*H 8 or pepsin at *p*H 3 results in complete inactivation within 60 min. at 37° C.

However, Cameron[2,3] has suggested that the active substance from the corpus cardiacum is an *o*-diphenol. Since Cameron's extraction technique involves the use of absolute ethanol as a solvent for the active principle, his material must be different from the material under study here. Further confirmation of the non-identity of the two materials is obtained from a consideration of the behaviour on paper chromatograms of distilled water extracts of corpora cardiaca and extracts prepared according to the method of Cameron. Using *n*-butanol/acetic acid/water (4 : 1 : 5) as a solvent, the activity from corpora cardiaca extracted in distilled water is associated with a ninhydrin-positive spot at R_F 0·23. Similar chromatograms using Cameron's technique have yielded highly variable results; it is sufficient to say here that no activity was ever recovered from the area around R_F 0·23. Further, a number of spots, some sensitive to ninhydrin, others to ferric ferricyanide, appeared at higher R_F values. None of these spots could be detected on chromatograms developed from distilled water extracts. Using phenol as a solvent, it has not been possible to detect a spot on chromatograms developed from distilled water extracts which turns pink after treatment with 0·44 *M* potassium ferricyanide. On the other hand, such spots appear on chromatograms in which Cameron's technique was used to extract the corpora cardiaca. It is worth noting that Cameron preferred to extract the *o*-diphenol from whole larvæ of *Tenebrio*, as the extraction from the corpora cardiaca of *Periplaneta* was difficult and unreliable[2].

The material from the pericardial cells appears to be an indolalkylamine. The isolated cockroach heart is very sensitive to serotonin; the threshold for stimulation is below 10^{-7} *M*. Extracts of pericardial cells activated by the factor from the corpus cardiacum have a slight and transient melanophorotropic effect on the melanocytes of the skin of the frog *Rana temporaria*, a property shared by the indolalkylamines[4]. The action of extracts of the corpus cardiacum on the isolated heart of *Periplaneta* can be potentiated by first exposing the heart to a concentration of 100 μgm./ml. of iproniazid, an inhibitor of the enzyme monoamine oxidase. Further, 2-bromolysergic acid diethylamide, an antagonist of indolalkylamines, markedly reduced the effect of extracts of corpus cardiacum when applied at a concentration of 10^{-5} *M*. These results are taken as evidence that an indolalkylamine is involved in the action of the corpus cardiacum on the heart.

If activated pericardial cells are extracted by a technique designed to separate amines[5], a pharmacologically potent extract results which possesses a single ultra-violet absorption peak at 266 mμ. Similar extracts of inactive pericardial cells, on the other hand, are pharmacologically inert, and possess an absorption peak at 275 mμ, with a shoulder at 286 mμ. This probably represents an inactive precursor of the active factor.

Extracts of active pericardial cells have been run on paper chromatograms using *n*-butanol/acetic acid/water (4 : 1 : 5) as a solvent. The pharmacological activity on such chromatograms is located in the vicinity of R_F 0·5. After treatment with ninhydrin-acetic[6], a spot exhibiting blue-green fluorescence in ultra-violet light appears at R_F 0·5. This spot is absent from chromatograms of extracts of inactive pericardial cells. The test is diagnostic for derivatives of tryptamine. It is worth noting that the active factor from the opaque accessory glands of male insects[7] can be extracted by the same technique and shows a strong absorption peak at 266 mμ.

Finally, active and inactive pericardial cells have been examined histologically. Active cells are considerably larger and contain more secretion droplets. A greater proportion of the active cells exhibit argentaffin granules, a characteristic feature of cells which produce indolalkylamines.

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