were submitted to the tests. On the line 3 cm. from the bottom, at 2-cm. intervals, 0.01 cc. each of 0.1 mg./cc. solution of the sample compound was spotted by a micropipet, and colored by the Dragendorff reagent, causing red spots to appear on a pale yellow background. When the spot was obscure, the strips were heated at 80° for 5 mins. and washed with water, by which the spots appeared clearly. The solutions were prepared in $CHCl_3$ for the compounds (4), (5), (6), (7), and (8), in water for (1), (2), and (10), in EtOH for (9) and (11), and in MeOH for (3), listed in Table I.

ii) Developing Medium : Upper layer or the respective mixtures was used, No. 1 after being allowed to stand for 3 days, No. 2 after 14 days, and No. 3 after 4 days.

Absorption Spectral Measurements—Shimadzu Spectrophotometer Type QB 50 was used. Usually, visible region from 203 mμ was measured. The solvents were purified by the usual method. Distilled water was used after two distillations. Quartz cell of 10-mm. optical depth was used. The intervals of measurement was ususlly 5~10 mμ or 0.5 mμ near the peaks.

Summary

In order to find out the changes in the acceleration of visual acuity in the dark by the administration of chemicals and their reaction mechanisms, compounds related to arecoline were synthesized.

(Received April 8, 1955)

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47. Takeo Tsukamoto and Tetsuya Komori : Studies on Visual Function. III.[1] Regeneration of Visual Purple by Allied Compounds of Arecoline.

(*Pharmaceutical Institute, Medical Faculty, University of Kyushu**)

Decomposition of rhodopsin by photo-impulsion is regenerated immediately after interception of the excitement and the rate of this regeneration depends on various conditions and factors. Change of regeneration rate by the administration of chemicals has been observed by many and pilocarpine is said to accelerate the reaction while cocaine suppresses it. Examinations have been made as to the effect of scopolamine, acetylcholine, arecoline, methionine, betaine, physostigmine, cysteine, cystine, and creatinine, but no systematic studies have been carried out.

In the previous paper,[1] syntheses of some allied compounds of arecoline were described. Observations as to their action mechanism will be deferred to a later date and, as a fundamental experiment, comparative examination of these compounds on the rhodopsin regeneration of the retina, as the change of the receptor alone, will be described below.

Methods

Toads (*Bufo vulgaris* formosus *Boulenger*) of constant weight, with identical external appearance and size of eyes, were chosen as the experimental animals. After light adaptation in the light adaptation apparatus, 0.01 mole of each of the sample compound was injected into the abdominal lymph sac of the toad as 0.6 cc./200 g. Ringer solution for cold-blooded animals, and the animals were immediately submitted to dark adaptation. The concentration of rhodopsin in the retina after 30 and 60 mins. was compared with those of the control animal given only the Ringer solution and after dark adaptation.

Operation in the Light—The light-adaptation apparatus was a white enamelled tank of 24 cm. in inside diameter and 24 cm. in height. About 1 cm. above the tank, a glass plate was placed and about 3 cm. above this glass plate, Mazda incandescent lamp of 300 W, 100 V., was placed, to light the inside of the tank uniformly. The bottom of the tank was provided with 4~5 cm. of flowing water and the internal temperature was kept at 18~20°. Usually, 3~4 toads were placed in the tank as one group and submitted to light adaptation for 2.5 hrs. under observation.

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* Katakasu, Fukuoka (塚本赳夫, 古森徹哉).

1) Part II : This Bulletin, **3**, 243(1955).

Operation in the Dark—After light adaptation and injection of the chemical, the toads were placed in a vase of 29 cm. in internal diameter and 29 cm. in depth and the vase was covered with a black cloth. After 30 or 60 mins., the animal was beheaded under a box provided with a frosted glass plate and containing a red lamp of 20 V., 2 candle power, at a distance of about 25 cm. from the animal. The eyeballs from the beheaded animal were cut into half and the retina taken out into a petri dish containg the Ringer solution. The retina from both eyes were combined and digested with 4 cc. of 2% sodium cholate for 5 hrs., with occasional shaking, centrifuged, and the supernatant was submitted to the measurement of optical density at 500 mμ, corresponding to the absorption maximum of rhodopsin. Shimadzu Type QB 50 spectrophotometer with the cell of Corex glass of 10 mm. optical depth was used. For the duration of about 2 mins. required for the measurement, rhodopsin did not undergo any change. The temperature of the dark room was maintained at 12~14°.

Experimental Results and Considerations

The absorption curve for rhodopsin was first shown by Kühne[2] and by Kottgen and Abelsdorff,[3] and through spectrophotometer by Chase,[4] Krause,[5] and by Hosoya and Saito.[6] Measurement of the spectrophotograph was made by Wald,[7] Lythgoe,[8] and Hosoya.[9]

The presence of the absorption maximum at 500 mμ, with the curve lying between 400 and 620 mμ, agrees with the observations made by the present authors, who made the measurements at 500 mμ.

Examination of the whole course of rhodopsin regeneration action of each chemical is limited by the number of animals of definite weight that can be obtaind. As shown by the summarized results in Table I, the comparison was made by taking the average values 0.160 and 0.212 of the five measurements of the retina of the control animal respectively at 30 and 60 minutes after the start of dark adaptation as the standards. In the arecaidine group, the esters effected acceleration of the rhodopsin regeneration while carboxylic acids effected suppresion. This is an interesting point in comparison to the work of Santoni[10] who measured Q_{O_2} of the retina and reported that fatty acids other than formic, capric, and oleic acids, especially their methyl esters rather than the saturated monocarboxylic acids, effected such action. Adminstration of 0.01 mole of arecoline methiodide resulted in marasmic symptoms and death in 6 out of 7 animals and 0.002 mole concentration showed the effect corresponding to that of arecoline. Of the esters, the methyl ester seems to have the strongest effect.

In compounds possessing a pyridine ring, contrary to the action of arecoline methiodide, nicotinamide methiodide and its ring hydrogenated compound showed no effect, in spite of the fact that nicotinamide itself showed acceleration effect. This is assumed to be due to the action of nicotinamide limited only as the factor of diphosphopyridine nucleotide (DPN). None of the pyridine-carboxylic acids, except picolinic acid methylbetaine, showed any effect. In general, some kinds of betaine are known to accelerate the action but the mechanism of such action is still obscure.

Whether the accelerative action of arecaidine esters is due to the unsaturated character of the piperidine skeleton or to the quaternary ammonium base must await further accumulation of data.

2) Kühne: "Hermann's Handbuch Physiol.," **III**, 270(1876).
3) Kottgen, Aborsdorf: Z. psychol. Sinnes org., **12**, 161(1896).
4) Chase: J. Gen. Physiol., **19**, 577(1936).
5) Krause, Sidwell: Am. J. Physiol., **121**, 215(1938).
6) Y. Hosoya, Z. Saito: Tohoku J. Exptl. Med., **29**, 172(1935), **32**, 399(1939).
7) Wald: J. Gen. Physiol., **21**, 795(1938).
8) Lythgoe: J. Physiol., **89**, 331(1937).
9) Y. Hosoya: Japan. J. Med. Sci., **4**, 49(1935).
10) Santoni: Ann. Ottalmol. e clin. oculist., **67**, 845(1939),

Table I. Optical Density at the Time of Dark Adaptation

Time in min.	Average Effect 30		60	
Control	0.166 0.162 0.157 0.154 0.161	0.160	0.225 0.200 0.215 0.205 0.215	0.212
Arecaidine Hydrobromide	0.165 0.155 0.165	0.161 −	0.205 0.205 0.210	0.206 −
Dihydroarecaidine hydrobromide	0.167 0.153 0.163	0.161 −	0.222 0.195 0.194	0.203 −
Methyl nicotinate	0.172 0.170 0.162	0.168 ±	0.210 0.216 0.228	0.218 ±
Nicotinamide	0.200 0.203 0.207	0.203 +	0.236 0.242 0.222 0.256	0.239 +
Nicotinamide methiodide	0.169 0.180 0.173	0.174 ±	0.216 0.206 0.223	0.215 ±
Methyl nicotinate Methiodide	0.180 0.163 0.169	0.170 ±	0.212 0.218 0.211	0.214 ±
Picolinic acid methyl-betaine hydriodide	0.206 0.216 0.190	0.204 +	0.247 0.240 0.231	0.239 +
Ethyl isonicotinate methiodide	0.162 0.167 0.170	0.166 ±	0.212 0.216 0.230	0.219 ±
Ethyl N-methylisonipecotinate hydriodide	0.195 0.167 0.157	0.172 ±	0.217 0.214 0.220	0.217 ±
N-Methylnipecotinamide hydriodide	0.183 0.169 0.162	0.171 ±	0.216 0.206 0.223	0.215 ±
Arecoline hydrochloride	0.183 0.212 0.220	0.205 +	0.264 0.227 0.223	0.248 +
Arecoline methiodide*	0·220 0.215 0.217 0.180	0.208 ++	0.252 0.218 0.230 0.275	0.244 ++
Arecaidine ethyl ester hydrochloride	0.184 0.200 0.193	0.192 +	0.235 0.236 0.230	0.230 +
Arecaidine propyl ester hydrochloride	0.193 0.197 0.190	0.193 +	0.224 0.224 0.264	0.237 +
Arecaidine isopropyl ester hydrochloride	0.190 0.195 0.200	0.195 +	0.245 0.245 0.230	0.240 +
Arecaidine butyl ester hydrochloride	0.182 0.212 0.197	0.197 +	0.243 0.235 0.220	0.233 +

* Inject., 0.002 mole

Two kinds of actions were assumed to have occurred in the regeneration of rhodopsin, i.e. the one occurring directly *in vitro* to activate retinene reductase oxidatively and indirectly *in vivo* to raise the functions of pigment layer and to accelerate blood circulation. It was found by the study of electroretinogram in detail, in cooperation with the Physiological Department, that the action of arecaidine esters was slight in the retinal tissue dissected as eye cup. The fact suggests that the action of arecaidine esters might be mostly a secondary action in stimulating nerve endings and in raising secretion. This work will be described in subsequent papers.

Conclusion

1. Arecoline methiodide showed the action equal to that of arecoline in 1/5 the concentration of the latter in accelerating rhodopsin regeneration.
2. Arecaidine and dihydroarecaidine suppresses the action.
3. Arecaidine esters, picolininic acid methylbetaine, and nicotinamide accelerate the action.
4. No effect was seen in methyl nicotinate, nicotinamide methiodide, methyl nicotinate methiodide, ethyl isonicotinate methiodide, ethyl N-methylisonipecotinate hydriodide, and N-methylnipecotinamide hydriodide.

The authors are deeply grateful to Prof. Y. Hosoya of the Physiological Department of Osaka City Medical College and Prof. N. Toida and Messrs. Ogoh and Kuriyama of the Physiological Department, University of Kyushu, for valuable advices. The animal experiments were carried out with the cooperation of Messrs. Yokoyama and Muraoka of this Institute to whom grateful acknowledgment is expressed.

Summary

In order to find the effect of allied compounds of arecoline on the rhodopsin regeneration *in vivo*, rhodopsin concentration in the retina was measured in toads allowed to stand in the dark for a definite time after administration of such compounds and the values were compared with those of the control animal treated in the same way.

(Received April 8, 1955)